DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

# Memorandum

Date MAR - 3 2000

3195 '00 MAR -7 P2:37

From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

| | |
|---|---|
| New Dietary Ingredient: | *Haematococcus pluvialis* algae |
| Firm: | Aquasearch, Inc. |
| Date Received by FDA: | December 16, 1999 |
| Amendment 1 Received: | February 11, 2000 |
| Amendment 2 Received: | February 22, 2000 |
| 90-day Date: | May 21, 2000 |

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after May 21, 2000.

Robert J. Moore, Ph.D.

95S-0316

RPT65

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

MAR - 3 2000 3196 '00 MAR -7 P2:37

Mr. Martin Guerin
V.P. Marketing & Sales
Aquasearch, Inc.
73-4460 Queen Kaahumanu Highway
Suite 110
Kailua-Kona, Hawaii 96740

Dear Mr. Guerin:

This is to notify you that your submission pursuant to section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act) dated December 6, 1999, concerning the marketing of a substance that you assert is a new dietary ingredient (i.e., *Haematococcus pluvialis* algal meal containing astaxanthin), was received by the Food and Drug Administration (FDA) on December 16, 1999. FDA received amendments to your submission on February 11 and February 22, 2000. On February 23, 2000, we notified you that your February 22, 2000 amendment was substantive and that its date of receipt would constitute the filing date for your submission.

Your submissions will be kept confidential for 90 days from the date of receipt, and after May 21, 2000, your submission will be placed on public display at Dockets Management Branch (Docket No. 95S-0316). Commercial and confidential information in the notification will not be made available to the public.

Please contact us if you have questions concerning this matter.

Sincerely,

Robert J. Moore, Ph.D.
Acting Chief, Dietary Supplements Branch
Division of Compliance and Enforcement
Office of Nutritional Products, Labeling,
and Dietary Supplements

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

FEB 23 2000

3197 '00 MAR -7 P2:37

Mr. Martin Guerin
V.P. Marketing & Sales
Aquasearch, Inc.
73-4460 Queen Kaahumanu Highway
Suite 110
Kailua-Kona, Hawaii 96740

Dear Mr. Guerin:

This is to notify you that the amendment, dated February 16, 2000, to your submission pursuant to section 413(a)(2) (21 U.S.C. 350b(a)(2)) of the Federal Food, Drug, and Cosmetic Act (the Act) concerning the marketing of a substance that you assert is a new dietary ingredient (i.e., Haematococcus algae) was received by the Food and Drug Administration (FDA) on February 22, 2000.

In accordance with 21 CFR 190.6(d), this letter is to notify you that FDA has determined that the new submission is a substantive amendment and, accordingly, the new filing date for your submission is February 22, 2000.

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement that contains a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. A dietary supplement that contains a new dietary ingredient is considered adulterated under 21 U.S.C. 342(f)(1)(B) if it is introduced into interstate commerce less than 75 days after submitting such a notification. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v). Therefore, you should not introduce a dietary supplement containing the new dietary ingredient that is the subject of your submission before 75 days after the new filing date for your submission.

Please contact us if you have questions concerning this matter.

Sincerely,

Robert J. Moore, Ph.D.
Acting Chief
Dietary Supplements Branch
Division of Compliance and Enforcement
Office of Nutritional Products, Labeling,
and Dietary Supplements

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington DC 20205

Aquasearch Inc.
bringing the oceans to life

Aquasearch Inc.
Kona Production Facility
73-4460 Queen Kaahumanu Highway
Suite 110, Kailua-Kona, HI 96740 USA
Tel: (808)326-9301 Fax: (808)326-9401
WebSite: http://www.aquasearch.com/
E-mail: aqse@aquasearch.com

December 6, 1999

Re: New Dietary Ingredient Notification for Haematococcus algae.

Dear Administrator,

According to rule 21 CFP Subpart B 190.6, we are sending you two notifications in the attached documents. The first is for a New Dietary Ingredient Notification for *Haematococcus pluvialis* algal meal (Section 1 and 2), and the second is a New Dietary Supplement Notification for The AstaFactor™ - Softgels – 5mg (Section 3 and 4). Throughout these two applications, terms such as *Haematococcus pluvialis, H. pluvialis, Haematococcus,* are to be considered equivalent and interchangeable.
Although these are two separate applications, since they share a large part of the supporting documentation (Sections 5 to 9), they have been regrouped in the same folder.

We plan to market both products 75 days after the acknowledgement of receipt of this notice, unless otherwise instructed by your agency.

Please find enclosed one original and two copies of these two notifications.

Thank you very much in advance for the attention that you will give to our notification. If you have any question, do not hesitate contacting us at 808-326 9301.

Sincerely yours,

Martin Guérin
V.P. Marketing & Sales
Aquasearch Inc.

000001

## TABLE OF CONTENTS: 75-DAYS PRE-MARKET NOTIFICATION TO FDA

Sections:



000002

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington, DC 20205

Aquasearch Inc.
bringing the oceans to life

Aquasearch Inc.
Kona Production Facility
73-4460 Queen Kaahumanu Highway
Suite 110, Kailua-Kona, HI 96740 USA
Tel: (808)326-9301 Fax: (808)326-9401
WebSite: http://www.aquasearch.com/
E-mail: aqsc@aquasearch.com

December 6, 1999

Re: New Dietary Ingredient Notification for *Haematococcus* algae.

Dear Administrator,

Pursuant to rule 21 CFP Subpart B 190.6, please be advised of this New Dietary Ingredient Notification for *Haematococcus pluvialis* algal meal.
Throughout this application, terms such as *Haematococcus pluvialis, H. pluvialis, Haematococcus,* are to be considered equivalent and interchangeable.

We plan to market *Haematococcus* algae as an ingredient for human dietary supplements, 75 days after the acknowledgement of receipt of this notice, unless otherwise instructed by your agency.

Please find enclosed one original and two copies of this notification.

We presently produce an algal meal prepared from *Haematococcus*, which we market as a feed ingredient in Europe and in Asia, for application in fish and poultry diets. This product is stabilized with ethoxyquin.

The product which we plan to market will be an algal meal prepared at our facility in Kona. It will not be stabilized with ethoxyquin but may be sold as is or stabilized with an approved natural food-grade antioxidant, such as natural vitamin E, grapeseed extract, or an equivalent approved antioxidant.

The algal meal may be used as an ingredient in tablets, capsules or other dietary supplement preparations. It may also be used as a base for further extraction with approved food-grade solvents and extraction processes, to recover some of the active components, such as natural astaxanthin and other pigments.

The algal meal will be manufactured by:

Aquasearch, Inc.
73-4460 Queen Kaahumanu Hwy., Suite #110,
Kailua-Kona, Hawaii 96740,
Tel: (808) 326 – 9301, Fax: (808) 326 – 9401

000003

The scientific name of the dietary ingredient subject to premarket notification, is the Latin specific name, *Haematococcus pluvialis*, or the Latin generic, *Haematococcus.*

A trademark may precede this generic scientific name, such as: Nutraxan™Asta or the AstaFactor™.

Information on the origin, manufacturing process and specifications, as well as proposed labeling and usage recommendations, has been provided in the attachments (Section 2).

A daily serving of 5mg astaxanthin and/or 250 mg algal meal, in one or two tablets or softgels is suggested.

There has been already significant information made public to support the safety of astaxanthin and of *Haematococcus* as a dietary supplement (see Section 5 for details), including a New Dietary Ingredient Notification for *Haematococcus* algae, from Cyanotech Corporation, submitted to FDA on March 18, 1999. In addition, antioxidant properties of astaxanthin and its metabolic effects have been widely investigated (see Section 6) and give no reason to believe that there should be any safety concern when ingested at the recommended levels (see Section 5 for details). We feel that Aquasearch's unique proprietary production technology and the well documented safety studies conducted on our product (Section 5), provide unique additional information to confirm that algal astaxanthin and, more broadly, *Haematococcus* produced with Aquasearch's technology, is safe when used as an ingredient for dietary supplements according to the appropriate recommendations.

We contend that this product is a natural product which is unadulterated and meets the evidence of safety under the Federal Food, Drug and Cosmetic Act sec. 413 (350b)(a)(2).

Furthermore the product is produced under strict quality control and according to current dietary supplement GMP recommendations, with quality checks throughout manufacturing and after processing.

Thank you very much in advance for the attention that you will give to our notification. If you have any question, do not hesitate contacting us at (808) 326-9301.

Sincerely yours,

Martin Guérin
V.P. Marketing & Sales
Aquasearch Inc.

000004

Technical report

# *Haematococcus pluvialis* algal meal produced with Aquasearch's proprietary technology: a unique source of natural astaxanthin and algal nutrients.

(TR.3004.001)

- ***Haematococcus pluvialis** is an ubiquitous, unicellular green alga that produces the carotenoid pigment, astaxanthin, when in the resting, or "haematocyst", stage.*
- ***To produce the meal, the green, swimming stages of H. pluvialis are cultivated in closed, tubular growth modules. When at sufficient concentrations, green cells are transferred to ponds where conditions favor the rapid transition into the red, resting stage.***
- ***Red haematocysts are harvested, dewatered, and mechanically broken to improve the bioavailability of astaxanthin. The resulting slurry is pasteurized and dried at moderate temperature into flakes which are ground up and mixed with a natural food grade antioxidant to stabilize the pigment. The algal meal is then packed into aluminum foil-lined polyethylene bags.***
- ***Haematococcus pluvialis algal meal is at least 2% astaxanthin, of which >80% is in the monoester form. The meal typically contains >18% protein and >19% fat, and has a low bacterial counts within standard human safety levels.***

## 1. Occurrence and life cycle of *Haematococcus pluvialis*.

***Haematococcus pluvialis* Flotow, 1844**
**Family: Haematococcaceae**
**Order: Volvocales**
**Class: Chlorophyceae**
**Division: Chlorophyta**

*Haematococcus pluvialis* is a flagellated unicellular green alga (Chlorophyte) that produces the red pigment, astaxanthin, during a resting or encysted stage induced by extreme environmental conditions. *H. pluvialis* was described in 1844, although what may be synonymous species were recognized much earlier (Table 1). It is widely distributed across Europe, Africa and North America, typically in small, ephemeral pools (Almgren 1966, Thompson & Wujek 1989). The striking red color it imparts to such pools led to extensive study, which continues to the present,



000005

Aquasearch Inc., ©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: (808)-326-9301, Fax: (808)-326-9401

of its natural history and production of red "haematochrome" (now known to be astaxanthin), which continues to the present.

*H. pluvialis* changes in morphology during a life cycle that is driven by environmental conditions (Elliot 1934, Droop 1953, 1954; Almgren 1966):

a) The large (8-50 μm diameter), flagellated green form or **macrozooid** is the most common in rapidly growing cultures. Macrozooids divide into other macrozooids, usually in the early morning.
b) As conditions deteriorate (e.g., slow desiccation, extremes in pH and strong illumination), macrozooids lose their flagella and become highly vacuolated, floating **palmellae** which remain green. Palmellae can divide into other palmellae, or into flagellated macrozooids.
c) Under nutrient-poor conditions, red **haematocysts** typically develop from palmellae that have ceased to divide, but occasionally may arise from macrozooids. Haematocysts have heavy cell walls and produce large amounts of astaxanthin, when in bright sunlight.
d) If conditions improve, haematocysts divide into small (~20 μm), cylindrical flagellated **microzooids** which eventually lose their flagella, enlarge, and become palmellae. More often, however, haematocysts divide into larger macrozooids and the cycle repeats.

Aquasearch has several existing and pending patents for its unique proprietary technology to grow and process *Haematococcus* algae. This proprietary technology ensures the highest quality of algal meal as a source of natural astaxanthin and other nutrients for use in dietary supplements.

## 2. Culturing *Haematococcus* algae (Fig. 1)

The life cycle of *Haematococcus* algae dictates how production of natural astaxanthin is optimized. At Aquasearch , Inc., the algae are grown in three phases:

a) Small-volume stock cultures maintained in the laboratory are scaled up in increasingly larger containers to produce inoculum for outdoor mass culture;
b) Continuous culture of macrozooids in large, tubular closed photobioreactors or modules under nutrient-replete conditions;
c) Reddening in ponds under nutrient-poor conditions.

In the first two phases, the emphasis is on obtaining high concentrations of *Haematococcus* over very short periods of time. Rapid cell proliferation is maintained in Aquasearch Growth Modules (AGMs) by periodically harvesting part of the culture into ponds and replacing this with fresh nutrient medium. In ponds, on the other hand, conditions are controlled to accelerate cell encystment and astaxanthin production. The ability to grow large concentrations of pure cultures of *Haematococcus pluvialis* in AGMs and to stock ponds with those high concentrations ensures a very rapid and synchronized reddening, minimizing the risk of contamination and maximizing astaxanthin production, while minimizing levels of astaxanthin intermediary metabolites and other carotenoids.

## 3. Processing of algal meal (Fig. 1)

When a pond culture has properly reddened, it is harvested, dewatered and rinsed. Cells in the resulting slurry are then mechanically broken to improve the release of the pigment. This process



000006

Aquasearch Inc., ©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: (808)-326-9301, Fax: (808)-326-9401

improves bioavailability of astaxanthin when ingested, since intact cyst walls are inefficiently digested (Sommer et al., 1994). This cell wall rupture process also helps deactivate other potentially occurring harmful micro-organisms. Cell-broken product is subsequently pasteurized and dried at moderate temperature to minimize degradation of astaxanthin and other nutrients, while reducing bacterial counts to levels safe for human consumption (Table 2). Because the product results from a natural growth process and is subject to natural variability, several drying batches may be subsequently mixed in order to achieve predetermined standard astaxanthin levels: 2%, 2.5% and 3%. The resulting dry flakes of one or several batches are then ground up to a powder and mixed with an approved food-grade natural antioxidant, which helps stabilize the pigment during storage. The algal meal is then packed in air-tight aluminum-lined polyethylene bags and stored until further processing.

## 4. Algal meal composition (Table 2)

*Haematococcus pluvialis* algal meal is a good source of protein, fat, astaxanthin and other micronutrients. It accumulates astaxanthin when stressed by environmental changes (Grung et al. 1992). The astaxanthin stereo-isomer deposited in *Haematococcus pluvialis* is the 3*S*,3'*S* form (Grung et al. 1992), which is the main stereo-isomer encountered in wild salmon and many other aquatic species (Turujman 1997, Aquasearch 1999a). Typically, the main form of astaxanthin accumulated in *H. pluvialis* is the esterified form: monoesters represent more than 80% of the total astaxanthin, while diesters represent approximately 10 to 15%. Small amount of free astaxanthin (less than 10% of total astaxanthin) are also found (Akvaforsk, 1999, Latasa, 1995). In addition to astaxanthin other carotenoids can be encountered in *Haematococcus pluvialis*. Recent analyses on a representative batch determined that astaxanthin represented 84% of total carotenoids (Akvaforsk, 1999). This confirms analyses by other investigators (Table 3), with astaxanthin usually representing more than 85% of total carotenoids. Other carotenoids (Table 3) detected in small amounts in *Haematoccus* algae include: beta-carotene (0 to 5%), lutein (1 to 11%), cantaxanthin (2 to 5%). Analyses of Aquasearch algal meal have detected mainly lutein and beta-carotene (Latasa, 1995), although up to 2% of total carotenoids may be occurring as cantaxanthin (Aquasearch 1999). Because the process control and proprietary production technology used by Aquasearch is aimed at maximising astaxanthin production, the level of other carotenoid pigments stays within 10 to 15% of total carotenoids content.
*Haematococcus* has been employed as a constituent of feeds for a variety of animals, with no negative effects observed on growth, survival, behaviour, physiology or biochemistry (Nakazoe et al. 1984; Schiedt et al. 1986; Storrebakken et al. 1987; Ito et al. 1989; Sommer et al. 1991, 1992; Choubert & Heinrich 1993). *Haematococcus pluvialis* has been fed at levels up to 6% in trout diets without any negative effects (Choubert & Heinrich 1993). There is no indication that *Haematococcus* spp. contain any chemical compounds that may have a deleterious effect on other organisms. Safety studies conducted by Aquasearch in a representative animal model and with human volunteers have demonstrated that astaxanthin dietary supplements formulated with *Haematococcus pluvialis*, grown and processed with Aquasearch proprietary technology, are safe for human consumption, when following suggested inclusion levels (Aquasearch, 1999b).

## 5. References

Almgren K. (1966). Ecology and distribution in Sweden of algae belonging to Haematococaceae. Svensk Botanisk Tidskrift. BD.60, H.1, 49-73.
Aquasearch. 1999a. Technical Report TR.3001.001. Astaxanthin in nature.



Aquasearch Inc., ©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: (808)-326-9301, Fax: (808)-326-9401

000067

Aquasearch. 1999b. Technical Report TR.3005.001. *Haematococcus pluvialis* an astaxanthin safety for human consumption.

Aquasearch. 1999c. Technical Report TR.1006.001.

Boussiba S., L. Fan, and A. Vonshak (1993). Enhancement and determination of astaxanthin accumulation in green alga *Haematococcus pluvialis*. Methods in enzomology, 213: 386-

Choubert G. and O. Heinrich (1993). Carotenoid pigments of the green alga *Haematococcus pluvialis* : assay on rainbow trout *Onchorhynchus mykiss*, pigmentation in comparison with synthetic astaxanthin and cantaxanthin.

Droop M.R. (1953). On the ecology of flagellates from some brackishand fresh water rockpools of Finland. Acta Botanica Fennica 51, Ed. By: Societas Pro Fauna et Flora Fennica. 52pp.

Elliot A.M. (1934). Morphology and life history of *Haematococcus pluvialis*. Archiv. Protistekunde, 82:250-272.

Fan L., A. Vonshak & S. Boussiba. 1994. Effect of temperature and irradiance on growth of *Haematococcus pluvialis* (Chlorophyceae). J. Phycol., 30:829-833.

Goodwin T.W., M. Jamikorn (1954). Studies in carotegenesis. II. Carotenoid synthesis in the alga *Haematococcus pluvialis*. Biochemical Journal, 57:376-381.

Grung M., M.L. D'Souza, M. Borowitzka, & S. Liaaen-Jensen. 1992. Algal Carotenoids 51. Secondary Carotenoids 2. *Haematococcus pluvialis* aplanospores as a source of (3S,3'S)-astaxanthin esters. J. Appl. Phycol. 4:165-171.

Latasa, 1995. Analysis report to Aquasearch.

Lorenz T. 1998. Cyanotech Naturose Technical Bulletin #011.

Harker M. & A.J. Young. 1995. Inhibition of astaxanthin synthesis in the green alga, *Haematococcus pluvialis*. Eur. J. Phycol., 30:179-187.

Koyabashi M., T. Kakizono & S. nagai. 1991. Astaxanthin production by a green alga, *Haematococcus pluvialis* accompanied with morphological changes in acetate media. J. ferment. Bioeng., 71(5):335-339.

Kobayashi M., T. Kakizono, K. Yamaguchi, N. Nishio and S. Nagai (1992a). Growth and astaxanthin formation of *Haematococcus pluvialis* in heterotrophic and mixotrophic conditions. Journal of fermentation and Bioengineering, 74:61-63.

Nakazoe J., S. Ishii, M. Kamimoto and M. Takeuchi (1984). Effects of supplemental carotenoid pigments on the carotenoid accumulation in young red seabream (*Chrysophyrys major*). Bull. Tokai. Reg. Fish. Res. Lab., 113:29-41.Proctor V.E. (1957): Some controlling factors in the distribution of *Haematococcus pluvialis*. – Ecology, 457-462.

Schiedt K., M. Vecchi and E. Glinz (1986). Astaxanthin and its metabolites in wild rainbow trout *Onchorhynchus mykiss* . Aquaculture, 94:79-88.

Sommer T.R., W.T. Potts and N.M. Morissy (1991). Utilisation of microalgal astaxanthin by rainbow trout (*Onchorhynchus mykiss*). Aquaculture, 94:79-88.

Sommer T.R., F.M.L.D. Souza and N.M. Morissy (1991). Pigmentation of adult rainbow trout, *Onchorhynchus mykiss*, using the green alga *Haematococcus pluvialis*. Aquaculture, 106:63-74.

Turujman S.A., W.G. Wamer, R.R. Wei, R.H. Albert. 1997. Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. J. AOAC. Int., 80(3):622-632.

Zlotnik I., A. Sukenik., & Z. Dubinsky. 1993. Physiological and photosynthetic changes during the formation of red aplanospores in the chlorophyte *Haematococcus pluvialis*. J. Phycol., 29:463-469.

Yuan J.-P., X.-D. Gong, & F. Chen. 1996. Separation and identification of astaxanthin esters and chlorophylls in *Haematococcus lacustris* by HPLC. Biotechnol.. Tech., 10(9):463-469.



Aquasearch Inc., ©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: (808)-326-9301, Fax: (808)-326-9401

Table 1. Possible synonyms for *Haematococcus pluvialis* Flotow, 1844 (Almgren, 1966).

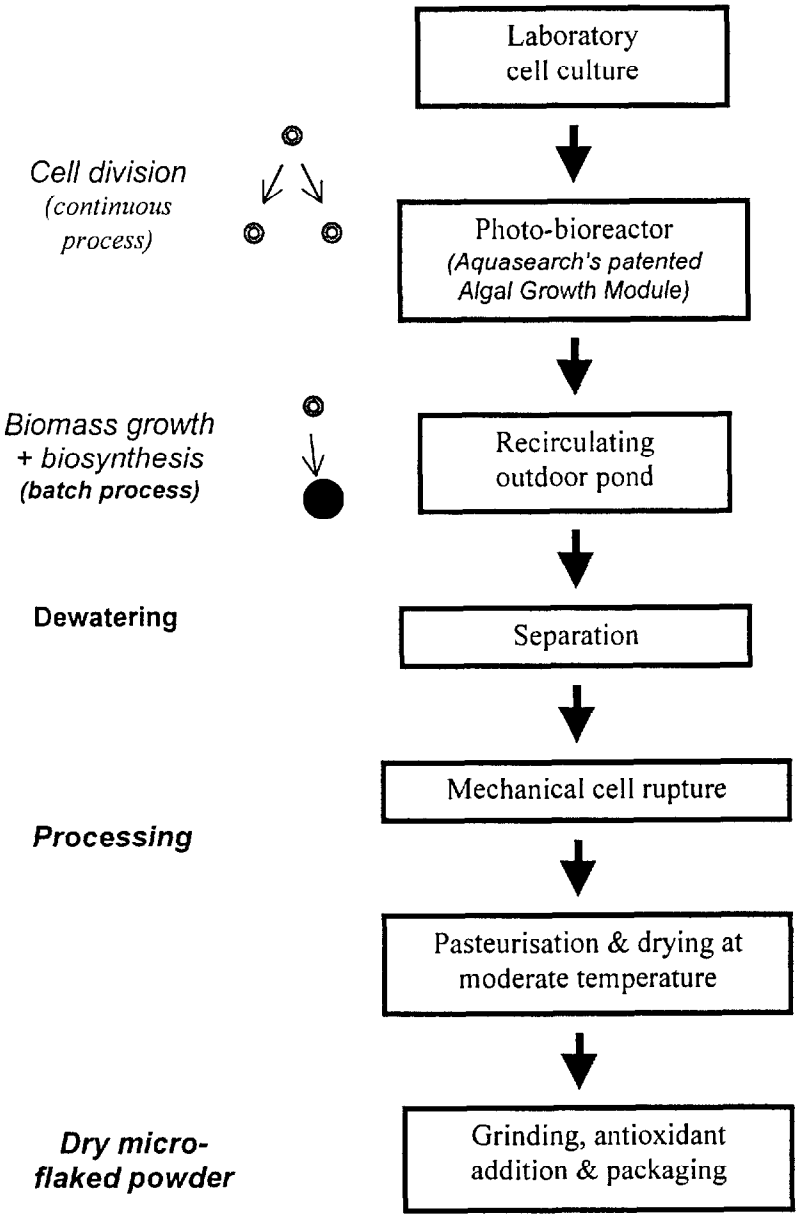
| Scientific names | Dates |
|---|---|
| *Volvox lacustris* Girod-Chantrans | 1802 |
| *Lepraria kermesina* Wrangel | 1824 |
| *Sphaerella Wranglelii* Sommerfelt | 1824 |
| *Protococcus nivalis* Agardh em. Greville | 1824 |
| *Chlorococcum kermesinum* (Wrangel) Fries | 1825 |
| *Byssus kermesina* (Wrangel) Wahlenberg | 1826 |
| *Haematococcus noltii* Agardh | 1828 |
| *Haematococcus Grevillei* Agardh | 1828 |
| *Protococcus monospermus* Gorda | 1833 |
| *Microcystis grevillei* (Agardh) Kutzing | 1833 |
| *Globulina kermesina* (Wrangel) Turpin | 1836 |
| *Discerea purpurea* A. et C. Morren | 1841 |
| *Protococcus cordae* Meneghini | 1843 |
| *Haematococcus pluvialis* Flotow | 1844 |
| *Protosphaera pluvialis* (Flotow) Trevisan | 1848 |
| *Protosphaera cordae* (Meneghini) Trevisan | 1848 |
| *Protococcus pluvialis* (Flotow) Kutzing | 1849 |
| *Chlamydococcus pluvialis* (Flotow) Braun | 1852 |
| *Hysginum pluviale* (Flotow) Perty | 1852 |
| *Haematococcus lacustris* (Girod-Chantrans) Rostafinski | 1875 |
| *Sphaerella pluvialis* (Flotow) Wittrock | 1896 |
| *Sphaerella lacustris* (Girod-Chantrans) Wittrock | 1896 |



Aquasearch Inc., ©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: (808)-326-9301, Fax: (808)-326-9401

000069

**Fig. 1. Aquasearch's production process for the algal meal prepared from the green algae: *Haematococcus pluvialis*.**






Aquasearch Inc., ©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: (808)-326-9301, Fax: (808)-326-9401

Table 2. Typical composition of Aquasearch's dried *Haematococcus pluvialis* algal meal.

| **Proximate analysis** | | |
|---|---|---|
| Crude protein | 18.08 | % |
| Crude fat | 19.43 | % |
| Ash | 3.28 | % |
| Crude fiber | 4.30 | % |
| Moisture | 3.54 | % |

| **Energy** | |
|---|---|
| Calories | 470 per 100 g |
| Calories from fat | 175 per 100 g |
| Calories from Saturated fat | 28 per 100 g |

| **Carbohydrates** | | |
|---|---|---|
| **Dietary fiber** | **26.5** | **%** |
| *Insoluble fiber* | *25.2* | *%* |
| *Soluble fiber* | *1.3* | *%* |
| **Sugars** | **0.77** | **%** |
| **Cholesterol** | **0** | **%** |

| **Amino acids** | | |
|---|---|---|
| Alanine | 7.48 | % |
| Arginine | 5.54 | % |
| Aspartic acid | 7.62 | % |
| Cystine/Cysteine | 0.90 | % |
| Glutamic acid | 9.60 | % |
| Glycine | 5.01 | % |
| Histidine | 1.52 | % |
| Isoleucine | 3.55 | % |
| Leucine | 7.73 | % |
| Lysine | 4.33 | % |
| Methionine | 1.5 | % |
| Phenylalanine | 3.56 | % |
| Proline | 4.83 | % |
| Serine | 4.77 | % |
| Threonine | 4.99 | % |
| Tryptophan | 1.60 | % |
| Tyrosine | 2.92 | % |
| Valine | 5.28 | % |
| Met + Cys | 2.39 | % |
| Met + tyr | 6.48 | % |

| **Micro-organisms** | |
|---|---|
| Aerobic plate count | < 1000 CFU/g |
| E. coli | < 10 CFU/g |
| Salmonella | Negative/25 g |

| **Heavy metals** | | |
|---|---|---|
| Lead | <0.5 | ppm |
| Mercury | <0.1 | ppm |
| Cadmium | <0.5 | ppm |
| Arsenic | <0.5 | ppm |

| **Carotenoids** | | |
|---|---|---|
| Total carotenoids | 2.6 | % |
| Total astaxanthin | 2.18 | % |
| *Free astaxanthin* | *3.6* | *%* |
| *Astaxanthin monoester* | *81.8* | *%* |
| *Astaxanthin diester* | *14.6* | *%* |

| **Fatty acids** | | |
|---|---|---|
| Caprylic acid | C-8:0 | <0.01 % |
| Capric acid | C-10:0 | <0.01 % |
| Lauric | C-12:0 | <0.01 % |
| Myristic | C-14:0 | 0.09 % |
| | C-14:1 | <0.01 % |
| Palmitic | C-16:0 | 2.82 % |
| Palmitoleic | C-16:1 | 0.11 % |
| Stearic | C-18:0 | 0.17 % |
| Oleic | C-18:1 | 3.28 % |
| Linoleic | C-18:2 | 3.11 % |
| γ linolenic ω-6 | C-18:3 | 1.89 % |
| Octadecatetraenoic | C-20:0 | 0.04 % |
| Gadoleic | C-20:1 | 0.03 % |
| **Total saturated fat:** | | **3.12 %** |
| **Tot.monosaturated fat:** | | **3.42 %** |
| **Tot.polyunsaturated fat:** | | **5.00 %** |

| **Vitamins** | | |
|---|---|---|
| Vitamin A | 22000 | IU/100 g |
| Alpha tocopherol | 412 | mcg/g* |
| Vitamin B6 | 0.14 | mg/100 g |
| Vitamin B12 | 0.04 | mg/100 g |
| Thiamine (B1) | 0.09 | mg/100 g |
| Riboflavin (B2) | 0.26 | mg/100 g |
| Niacin | 0.45 | mg/100 g |
| Folic acid | 0.39 | mg/100 g |
| Pantothenic acid | 2.47 | mg/100 g |
| Vitamin C | 0 | mg/100 g |

***(* before addition of any antioxidant)***

| **Minerals** | | |
|---|---|---|
| Calcium | 890 | ppm |
| Phosphorous | 3900 | ppm |
| Potassium | 2300 | ppm |
| Sodium | 2400 | ppm |
| Magnesium | 1500 | ppm |
| Iron | 880 | ppm |
| Cobalt | 0.58 | ppm |
| Nickel | 5.7 | ppm |
| Selenium | <0.5 | ppm |
| Molybdenum | <0.5 | ppm |
| Zinc | 49 | ppm |
| Chromium | 5.1 | ppm |



Aquasearch Inc., ©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: (808)-326-9301, Fax: (808)-326-9401

000011

**Table 3. Carotenoids content and distribution in *Haematococcus pluvialis* cysts**

| | Individual carotenoid pigments measured as % of total carotenoids | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Sources:** | A | B | C | D | E | F | G | H* | K* | **Range** |
| Carotenoid pigments | | | | | | | | | | |
| Astaxanthin (total) | 89.0 | 84.7 | >79.2 | 84-89 | 81.0 | 81.5 | 85.0 | 95.4 | 83.8 | **81 – 95** |
| *Free astaxanthin* | *1.0* | *n.a.* | *n.a.* | | *1.0* | *n.a.* | *5.0* | *2.4* | *2.06* | ***1-5*** |
| *Astaxanthin monoesters* | *49.0* | *n.a.* | *79.2* | *69-74* | *46.0* | *n.a.* | *70.0* | *83.2* | *68.6* | ***46-79*** |
| *Astaxanthin diesters* | *39.0* | *n.a.* | *n.a.* | *10-20* | *34.0* | *n.a.* | *10.0* | *9.8* | *12.2* | ***10-39*** |
| Beta-carotene | 3.0 | <1.3 | *n.a.* | | 5.0 | | *n.a.* | | *n.a.* | **0-5** |
| Lutein | 1.0 | 9.8 | *n.a.* | 1-9 | 6.0 | 10.6 | *n.a.* | 3.6 | *n.a.* | **1-11** |
| Cantaxanthin | 2.0 | 5.5 | *n.a.* | | 4.0 | | *n.a.* | | *n.a.* | **0-5.5** |
| Other carotenoids | 5.0 | <1.3 | *n.a.* | 6-9 | 4.0 | | *n.a.* | 0.9 | *n.a.* | **1-9** |

*Sources: A = Harker & Young (1995), B = Fan et al. (1995), C = Yuan et al. (1996), D = Koyabashi et al. (1991), E = Grung et al. (1992), F = Zlotnik et al. (1993), G = Lorenz (1998), H = Latasa (1995), I = Akvaforsk (1999)*
** = Aquasearch's Haematococcus algal meal. N.a. = not analyzed.*



Aquasearch Inc., ©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: (808)-326-9301, Fax: (808)-326-9401

000012

# DATASHEET

Aquasearch Inc.
bringing the oceans to life

## NUTRAXAN™ ASTA

Dried *Haematococcus pluvialis* algal meal

**DESCRIPTION AND USE:**

NUTRAXAN™ASTA is a dried algal meal prepared from *Haematococcus pluvialis* algae. After harvest, the algae are cell-broken, gently dried, stabilised with a natural antioxidant, and packed in heat-sealed, air-tight, aluminum-lined, polyethylene bags. During the processing they also undergo a pasteurization step to maintain a low bacterial count. NUTRAXAN™ASTA is a very good source of **natural astaxanthin, a superior biological antioxidant**, and of other micro-algal nutrients. Several grades of NUTRAXAN™ASTA are available, based on the guaranteed total astaxanthin content: Minimum 2.0%, 2.5% or 3.0%.

NUTRAXAN™ASTA is to be used as an ingredient for dietary supplements. Recommended inclusion rate: 250 mg or 5 mg astaxanthin per daily serving.

NUTRAXAN™ASTA has been produced under Aquasearch's strict manufacturing standards and proprietary technology for algae culture and processing, aimed at maximising nutrient content and availability, while ensuring that potential contaminants hazardous to health are maintained below accepted safe levels.

**TYPICAL COMPOSITION**

| Proximate analysis | | |
|---|---|---|
| Crude protein | 18.08 | % |
| Crude fat | 19.43 | % |
| Ash | 3.28 | % |
| Crude fiber | 4.30 | % |
| Moisture | 3.54 | % |

| Carotenoids | | |
|---|---|---|
| Total carotenoids | 2.6 | % |
| Total astaxanthin | 2.18 | % |
| *Free astaxanthin* | *3.6* | % |
| *Astaxanthin monoester* | *87.2* | % |
| *Astaxanthin diester* | *14.6* | % |

| Energy | |
|---|---|
| Calories | 470 per 100 g |
| Calories from fat | 175 per 100 g |
| Calories from saturated fat | 28 per 100 g |

| | | |
|---|---|---|
| **Carbohydrates** | 55.67 | % |
| **Dietary fiber** | **26.5** | **%** |
| *Insoluble fiber* | *25.2* | % |
| *Soluble fiber* | *1.3* | % |
| **Sugars** | **0.77** | **%** |
| **Cholesterol** | **0** | **%** |

| Fatty acids | | |
|---|---|---|
| Caprylic acid | C-8:0 | <0.01 % |
| Capric acid | C-10:0 | <0.01 % |
| Lauric | C-12:0 | <0.01 % |
| Myristic | C-14:0 | 0.09 % |
| Myristoleic | C-14:1 | <0.01 % |
| Palmitic | C-16:0 | 2.82 % |
| Palmitoleic | C-16:1 | 0.11 % |
| Stearic | C-18:0 | 0.17 % |
| Oleic | C-18:1 | 3.28 % |
| Linoleic | C-18:2 | 3.11 % |
| γ linolenic ω-6 | C-18:3 | 1.89 % |
| Octadecatetraenoic | C-20:0 | 0.04 % |
| Gadoleic | C-20:1 | 0.03 % |

| | |
|---|---|
| **Total saturated fat:** | **3.12 %** |
| **Tot.monosaturated fat:** | **3.42 %** |
| **Tot.polyunsaturated fat:** | **5.00 %** |



AQUASEARCH, INC., ©
73-4460 Queen Kaahumanu Hwy.,
Suite 110, Kailua-Kona, HI 96740, USA,

000013

# DATASHEET

Aquasearch Inc.
bringing the oceans to life

## Amino acids

| | | | | | |
|---|---|---|---|---|---|
| Alanine | 7.48 | % | Phenylalanine | 3.56 | % |
| Arginine | 5.54 | % | Proline | 4.83 | % |
| Aspartic acid | 7.62 | % | Serine | 4.77 | % |
| Cystine/Cysteine | 0.90 | % | Threonine | 4.99 | % |
| Glutamic acid | 9.60 | % | Tryptophan | 1.60 | % |
| Glycine | 5.01 | % | Tyrosine | 2.92 | % |
| Histidine | 1.52 | % | Valine | 5.28 | % |
| Isoleucine | 3.55 | % | Met + Cys | 2.39 | % |
| Leucine | 7.73 | % | Met + Tyr | 6.48 | % |
| Lysine | 4.33 | % | | | |
| Methionine | 1.50 | % | | | |

## Minerals

| | | |
|---|---|---|
| Calcium | 890 | ppm |
| Phosphorous | 3900 | ppm |
| Potassium | 2300 | ppm |
| Sodium | 2400 | ppm |
| Magnesium | 1500 | ppm |
| Iron | 880 | ppm |
| Cobalt | 0.58 | ppm |
| Nickel | 5.7 | ppm |
| Selenium | <0.5 | ppm |
| Molybdenum | <0.5 | ppm |
| Zinc | 49 | ppm |
| Chromium | 5.1 | ppm |

## Vitamins

| | | |
|---|---|---|
| Vitamin A | 22000 | IU/100 g |
| Alpha tocopherol | 412 | mcg/g* |
| Vitamin B6 | 0.14 | mg/100 g |
| Vitamin B12 | 0.04 | mg/100 g |
| Thiamine (B1) | 0.09 | mg/100 g |
| Riboflavin (B2) | 0.26 | mg/100 g |
| Niacin | 0.45 | mg/100 g |
| Folic acid | 0.39 | mg/100 g |
| Pantothenic acid | 2.47 | mg/100 g |
| Vitamin C | 0 | mg/100 g |

*(* before addition of any antioxidant)*

## Heavy metals

| | | |
|---|---|---|
| Lead | <0.5 | ppm |
| Mercury | <0.1 | ppm |
| Cadmium | <0.5 | ppm |
| Arsenic | <0.5 | ppm |

## Microorganisms

| | |
|---|---|
| Aerobic plate count | < 1000 CFU/g |
| *E. coli* | < 10 CFU/g |
| *Salmonella* | Negative/25 g |



AQUASEARCH, INC., ©
73-4460 Queen Kaahumanu Hwy.,
Suite 110, Kailua-Kona, HI 96740, USA,

000014

Aquasearch Inc.
bringing the oceans to life

# NUTRAXAN™ASTA

Ingredient for dietary supplements.
Source of natural astaxanthin, biological antioxidant.

Net weight: 10-kg Lot #:
Manufacturing date:
Use by: when stored unopened at 20°C
*Manufactured by:*
AQUASEARCH, INC., Kona Production Facility,73-4460 Queen Kaahumanu Hwy., Suite 110, Kailua-Kona, HI 96740, USA.

## Supplement facts

| | | | |
|---|---|---|---|
| **Daily serving size:** | 250 | mg | |
| **Servings per bag:** | 40,000 | | |
| **Amount per serving:** | | Unit | % daily value |
| Total calories | 1.17 | cal | 0% |
| Total fat | 48 | mg | 0% |
| Polyunsaturated fat | 12 | mg | n.a. |
| Sodium | 1.3 | mg | 0% |
| Total carbohydrate | 138 | mg | 0% |
| Protein | 45 | mg | 0% |
| Vitamin A | 55 | IU | 1% |
| Alpha-tocopherol | 0.1(0.9)* | mg | 1% (9%)* |
| Total carotenoids | 6 | mg | n.a.** |
| **Total natural astaxanthin** | **5** | **mg** | n.a.** |

Ingredients:
Algal meal (*Haematococcus pluvialis*), natural antioxidant (natural vitamin E and/or grapeseed extract).
** 9% if natural Vitamin E added as antioxidant, 1% if no Vitamin E added .*
*** n.a.= not applicable.*

000015

LBLntrx, 12/06/99

# DATASHEET

Aquasearch Inc.
bringing the oceans to life

## Dried *Haematococcus pluvialis* algal meal

**DESCRIPTION AND USE:**

Aquasearch's **Dried *Haematococcus pluvialis* algal meal** is prepared from *Haematococcus pluvialis* algae. After harvest, the algae are cell-broken, gently dried, stabilised with a natural antioxidant, and packed in heat-sealed, air-tight, aluminum-lined, polyethylene bags. During the processing they also undergo a pasteurisation step to maintain a low bacterial count. **Dried *Haematococcus pluvialis* algal meal** is a very good source of **natural astaxanthin, a superior biological antioxidant**, and of other micro-algal nutrients. Several grades of **Dried *Haematococcus pluvialis* algal meal** are available, based on the guaranteed total astaxanthin content: Minimum 2.0%, 2.5% or 3.0%.

**Dried *Haematococcus pluvialis* algal meal** is to be used as an ingredient for dietary supplements. Recommended inclusion rate: 250 mg or 5 mg astaxanthin per daily serving.

**Dried *Haematococcus pluvialis* algal meal** has been produced under Aquasearch's strict manufacturing standards and proprietary technology for algae culture and processing, aimed at maximizing nutrient content and availability, while ensuring that potential contaminants hazardous to health are maintained below accepted safe levels.

**TYPICAL COMPOSITION**

| **Proximate analysis** | | |
|---|---|---|
| Crude protein | 18.08 | % |
| Crude fat | 19.43 | % |
| Ash | 3.28 | % |
| Crude fiber | 4.30 | % |
| Moisture | 3.54 | % |

| **Carotenoids** | | |
|---|---|---|
| Total carotenoids | 2.6 | % |
| Total astaxanthin | 2.18 | % |
| *Free astaxanthin* | *3.6* | % |
| *Astaxanthin monoester* | *87.2* | % |
| *Astaxanthin diester* | *14.6* | % |

| **Energy** | | |
|---|---|---|
| Calories | 470 per 100 g | |
| Calories from fat | 175 per 100 g | |
| Calories from saturated fat | 28 per 100 g | |
| **Carbohydrates** | 55.67 | % |
| **Dietary fiber** | **26.5** | **%** |
| *Insoluble fiber* | *25.2* | % |
| *Soluble fiber* | *1.3* | % |
| **Sugars** | **0.77** | **%** |
| **Cholesterol** | **0** | **%** |

| **Fatty acids** | | |
|---|---|---|
| Caprylic acid | C-8:0 | <0.01 % |
| Capric acid | C-10:0 | <0.01 % |
| Lauric | C-12:0 | <0.01 % |
| Myristic | C-14:0 | 0.09 % |
| Myristoleic | C-14:1 | <0.01 % |
| Palmitic | C-16:0 | 2.82 % |
| Palmitoleic | C-16:1 | 0.11 % |
| Stearic | C-18:0 | 0.17 % |
| Oleic | C-18:1 | 3.28 % |
| Linoleic | C-18:2 | 3.11 % |
| γ linolenic ω-6 | C-18:3 | 1.89 % |
| Octadecatetraenoic | C-20:0 | 0.04 % |
| Gadoleic | C-20:1 | 0.03 % |

**Total saturated fat: 3.12 %**
**Tot.monosaturated fat: 3.42 %**
**Tot.polyunsaturated fat: 5.00 %**


AQUASEARCH, INC., ©
73-4460 Queen Kaahumanu Hwy.,
Suite 110, Kailua-Kona, HI 96740, USA,


000016

# DATASHEET

Aquasearch Inc.
bringing the oceans to life

## Amino acids

| | | | | | |
|---|---|---|---|---|---|
| Alanine | 7.48 | % | Phenylalanine | 3.56 | % |
| Arginine | 5.54 | % | Proline | 4.83 | % |
| Aspartic acid | 7.62 | % | Serine | 4.77 | % |
| Cystine/Cysteine | 0.90 | % | Threonine | 4.99 | % |
| Glutamic acid | 9.60 | % | Tryptophan | 1.60 | % |
| Glycine | 5.01 | % | Tyrosine | 2.92 | % |
| Histidine | 1.52 | % | Valine | 5.28 | % |
| Isoleucine | 3.55 | % | Met + Cys | 2.39 | % |
| Leucine | 7.73 | % | Met + Tyr | 6.48 | % |
| Lysine | 4.33 | % | | | |
| Methionine | 1.50 | % | | | |

## Minerals

| | | |
|---|---|---|
| Calcium | 890 | ppm |
| Phosphorous | 3900 | ppm |
| Potassium | 2300 | ppm |
| Sodium | 2400 | ppm |
| Magnesium | 1500 | ppm |
| Iron | 880 | ppm |
| Cobalt | 0.58 | ppm |
| Nickel | 5.7 | ppm |
| Selenium | <0.5 | ppm |
| Molybdenum | <0.5 | ppm |
| Zinc | 49 | ppm |
| Chromium | 5.1 | ppm |

## Vitamins

| | | |
|---|---|---|
| Vitamin A | 22000 | IU/100 g |
| Alpha tocopherol | 412 | mcg/g* |
| Vitamin B6 | 0.14 | mg/100 g |
| Vitamin B12 | 0.04 | mg/100 g |
| Thiamine (B1) | 0.09 | mg/100 g |
| Riboflavin (B2) | 0.26 | mg/100 g |
| Niacin | 0.45 | mg/100 g |
| Folic acid | 0.39 | mg/100 g |
| Pantothenic acid | 2.47 | mg/100 g |
| Vitamin C | 0 | mg/100 g |

*(* before addition of any antioxidant)*

## Heavy metals

| | | |
|---|---|---|
| Lead | <0.5 | ppm |
| Mercury | <0.1 | ppm |
| Cadmium | <0.5 | ppm |
| Arsenic | <0.5 | ppm |

## Microorganisms

| | |
|---|---|
| Aerobic plate count | < 1000 CFU/g |
| *E. coli* | < 10 CFU/g |
| *Salmonella* | Negative/25 g |


AQUASEARCH, INC., ©
73-4460 Queen Kaahumanu Hwy.,
Suite 110, Kailua-Kona, HI 96740, USA,


000017

Aquasearch Inc.
bringing the oceans to life

# *Haematococcus pluvialis* algal meal

Ingredient for dietary supplements.
Source of natural astaxanthin, biological antioxidant.

Net weight: 10-kg Lot #:
Manufacturing date:
Use by: when stored unopened at 20°C
*Manufactured by:*
AQUASEARCH, INC., Kona Production Facility,73-4460 Queen Kaahumanu Hwy., Suite 110, Kailua-Kona, HI 96740, USA.

## Supplement facts

| | | | |
|---|---|---|---|
| **Daily serving size:** | 250 | mg | |
| **Servings per bag:** | 40,000 | | |
| **Amount per serving:** | | Unit | % daily value |
| Total calories | 1.17 | cal | 0% |
| Total fat | 48 | mg | 0% |
| Polyunsaturated fat | 12 | mg | n.a. |
| Sodium | 1.3 | mg | 0% |
| Total carbohydrate | 138 | mg | 0% |
| Protein | 45 | mg | 0% |
| Vitamin A | 55 | IU | 1% |
| Alpha-tocopherol | 0.1 (0.9)* | mg | 1% (9%)* |
| Total carotenoids | 6 | mg | n.a.* |
| **Total natural astaxanthin** | **5** | **mg** | n.a.* |

Ingredients:
Algal meal (*Haematococcus pluvialis*), natural antioxidant (natural vitamin E and/or grapeseed extract).

** 9% if natural Vitamin E added as antioxidant, 1% if no Vitamin E added .*
*** n.a.= not applicable.*

000018

LBLam, 12/06/99

# DATASHEET

Aquasearch Inc.
bringing the oceans to life

## The AstaFactor™ algal meal

Dried *Haematococcus pluvialis* algal meal

**DESCRIPTION AND USE:**

**The AstaFactor™ algal meal** is a dried algal meal prepared from *Haematococcus pluvialis* algae. After harvest, the algae are cell-broken, gently dried, stabilised with a natural antioxidant, and packed in heat-sealed, air-tight, aluminum-lined, polyethylene bags. During the processing they also undergo a pasteurisation step to maintain a low bacterial count. **The AstaFactor™ algal meal** is a very good source of **natural astaxanthin, a superior biological antioxidant**, and of other micro-algal nutrients. Several grades of The AstaFactor™ algal meal are available, based on the guaranteed total astaxanthin content: Minimum 2.0%, 2.5% or 3.0%.

**The AstaFactor™ algal meal** is to be used as an ingredient for dietary supplements. Recommended inclusion rate: 250 mg or 5 mg astaxanthin per daily serving.

**The AstaFactor™ algal meal** has been produced under Aquasearch's strict manufacturing standards and proprietary technology for algae culture and processing, aimed at maximising nutrient content and availability, while ensuring that potential contaminants hazardous to health are maintained below accepted safe levels.

**TYPICAL COMPOSITION**

| Proximate analysis | | |
|---|---|---|
| Crude protein | 18.08 | % |
| Crude fat | 19.43 | % |
| Ash | 3.28 | % |
| Crude fiber | 4.30 | % |
| Moisture | 3.54 | % |

| Carotenoids | | |
|---|---|---|
| Total carotenoids | 2.6 | % |
| Total astaxanthin | 2.18 | % |
| *Free astaxanthin* | *3.6* | % |
| *Astaxanthin monoester* | *87.2* | % |
| *Astaxanthin diester* | *14.6* | % |

| Energy | |
|---|---|
| Calories | 470 per 100 g |
| Calories from fat | 175 per 100 g |
| Calories from Saturated fat | 28 per 100 g |

| | | |
|---|---|---|
| **Carbohydrates** | 55.67 | % |
| **Dietary fiber** | **26.5** | **%** |
| *Insoluble fiber* | *25.2* | % |
| *Soluble fiber* | *1.3* | % |
| **Sugars** | **0.77** | **%** |
| **Cholesterol** | **0** | **%** |

| Fatty acids | | | |
|---|---|---|---|
| Caprylic acid | C-8:0 | <0.01 % | |
| Capric acid | C-10:0 | <0.01 % | |
| Lauric | C-12:0 | <0.01 % | |
| Myristic | C-14:0 | 0.09 % | |
| Myristoleic | C-14:1 | <0.01 % | |
| Palmitic | C-16:0 | 2.82 % | |
| Palmitoleic | C-16:1 | 0.11 % | |
| Stearic | C-18:0 | 0.17 % | |
| Oleic | C-18:1 | 3.28 % | |
| Linoleic | C-18:2 | 3.11 % | |
| γ linolenic ω-6 | C-18:3 | 1.89 % | |
| Octadecatetraenoic | C-20:0 | 0.04 % | |
| Gadoleic | C-20:1 | 0.03 % | |
| **Total saturated fat:** | | **3.12 %** | |
| **Tot.monosaturated fat:** | | **3.42 %** | |
| **Tot.polyunsaturated fat:** | | **5.00 %** | |



AQUASEARCH, INC., ©
73-4460 Queen Kaahumanu Hwy.,
Suite 110, Kailua-Kona, HI 96740, USA,



000019

# DATASHEET

Aquasearch Inc.
bringing the oceans to life

## Amino acids

| | | | | | |
|---|---|---|---|---|---|
| Alanine | 7.48 | % | Phenylalanine | 3.56 | % |
| Arginine | 5.54 | % | Proline | 4.83 | % |
| Aspartic acid | 7.62 | % | Serine | 4.77 | % |
| Cystine/Cysteine | 0.90 | % | Threonine | 4.99 | % |
| Glutamic acid | 9.60 | % | Tryptophan | 1.60 | % |
| Glycine | 5.01 | % | Tyrosine | 2.92 | % |
| Histidine | 1.52 | % | Valine | 5.28 | % |
| Isoleucine | 3.55 | % | Met + Cys | 2.39 | % |
| Leucine | 7.73 | % | Met + Tyr | 6.48 | % |
| Lysine | 4.33 | % | | | |
| Methionine | 1.50 | % | | | |

## Minerals

| | | |
|---|---|---|
| Calcium | 890 | ppm |
| Phosphorous | 3900 | ppm |
| Potassium | 2300 | ppm |
| Sodium | 2400 | ppm |
| Magnesium | 1500 | ppm |
| Iron | 880 | ppm |
| Cobalt | 0.58 | ppm |
| Nickel | 5.7 | ppm |
| Selenium | <0.5 | ppm |
| Molybdenum | <0.5 | ppm |
| Zinc | 49 | ppm |
| Chromium | 5.1 | ppm |

## Vitamins

| | | |
|---|---|---|
| Vitamin A | 22000 | IU/100 g |
| Alpha tocopherol | 412 | mcg/g* |
| Vitamin B6 | 0.14 | mg/100 g |
| Vitamin B12 | 0.04 | mg/100 g |
| Thiamine (B1) | 0.09 | mg/100 g |
| Riboflavin (B2) | 0.26 | mg/100 g |
| Niacin | 0.45 | mg/100 g |
| Folic acid | 0.39 | mg/100 g |
| Pantothenic acid | 2.47 | mg/100 g |
| Vitamin C | 0 | mg/100 g |

*(* before addition of any antioxidant)*

## Heavy metals

| | | |
|---|---|---|
| Lead | <0.5 | ppm |
| Mercury | <0.1 | ppm |
| Cadmium | <0.5 | ppm |
| Arsenic | <0.5 | ppm |

## Microorganisms

| | |
|---|---|
| Aerobic plate count | < 1000 CFU/g |
| *E. coli* | < 10 CFU/g |
| *Salmonella* | Negative/25 g |



AQUASEARCH, INC., ©
73-4460 Queen Kaahumanu Hwy.,
Suite 110, Kailua-Kona, HI 96740, USA,

000020

Aquasearch Inc.
bringing the oceans to life

# The AstaFactor algal meal

Ingredient for dietary supplements.
Source of natural astaxanthin, biological antioxidant.

Net weight: 10-kg Lot #:
Manufacturing date:
Use by: when stored unopened at 20°C
*Manufactured by:*
AQUASEARCH, INC., Kona Production Facility,73-4460 Queen Kaahumanu Hwy., Suite 110, Kailua-Kona, HI 96740, USA.

## Supplement facts

| | | | |
|---|---|---|---|
| **Daily serving size:** | 250 | mg | |
| **Servings per bag:** | 40,000 | | |
| **Amount per serving:** | | Unit | % daily value |
| Total calories | 1.17 | cal | 0% |
| Total fat | 48 | mg | 0% |
| Polyunsaturated fat | 12 | mg | n.a. |
| Sodium | 1.3 | mg | 0% |
| Total carbohydrate | 138 | mg | 0% |
| Protein | 45 | mg | 0% |
| Vitamin A | 55 | IU | 1% |
| Alpha-tocopherol | 0.9 | mg | 9% |
| Total carotenoids | 6 | mg | n.a.* |
| **Total natural astaxanthin** | **5** | **mg** | n.a.* |

Ingredients:
Algal meal (*Haematococcus pluvialis*), natural antioxidant (natural vitamin E).
* n.a.= not applicable.

000021

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington DC 20205

Aquasearch Inc.
bringing the oceans to life

Aquasearch Inc.
Kona Production Facility
73-4460 Queen Kaahumanu Highway
Suite 110, Kailua-Kona, HI 96740 USA
Tel: (808)326-9301 Fax: (808)326-9401
WebSite: http://www.aquasearch.com/
E-mail: aqse@aquasearch.com

December 6, 1999

Re: New Dietary Ingredient Notification for *Haematococcus* algal meal. Stability studies

Dear Administrator,

As part of our New Dietary Ingredient Notification for *Haematococcus* algal meal, we have started stability studies (see section 7), which will support the shelf-life and "use by" recommendations that will appear on the label (see section 2).

Before the end of the 75-day period after notification we will send you an update on the progress of these studies.

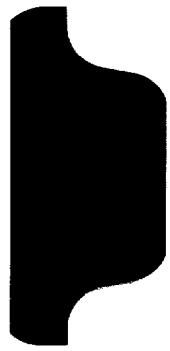
At this stage, our preliminary study shows only limited degradation of astaxanthin when the algal meal has been stabilized with a natural antioxidant and stored at 20°C for 6 months. Other internal studies have shown that no degradation in astaxanthin occurs during storage for 12 months at -20°C. This should allow for a shelf-life of at least 6 months at room temperature, and 12 months at -20°C.

Thank you very much in advance for the attention that you will give to our notification. If you have any question, do not hesitate contacting us at (808)326-9301.

Sincerely yours,

Martin Guerin
V.P. Marketing & Sales
Aquasearch Inc.

000022



Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington, DC 20205


bringing the oceans to life

Aquasearch Inc.
Kona Production Facility
73-4460 Queen Kaahumanu Highway
Suite 110, Kailua-Kona, HI 96740 USA
Tel: (808)326-9301 Fax: (808)326-9401
WebSite: http://www.aquasearch.com/
E-mail: aqse@aquasearch.com

December 6, 1999

Re: New Dietary Supplement Notification for The Astafactor ™, softgels, 5-mg.

Dear Administrator,

Pursuant to rule 21 CFP Subpart B 190.6, please be advised of this New Dietary Supplement Notification for a new dietary supplement: The Astafactor ™, softgels, 5-mg, formulated with the new dietary ingredient *Haematococcus* algal meal, for which Aquasearch is sending to FDA a separate New Dietary Ingredient Notification.

We plan to market The Astafactor ™, softgels, 5 mg, as a new human dietary supplement, 75 days after the acknowledgement of receipt of this notice, unless otherwise instructed by your agency.

Please find enclosed one original and two copies of this notification.

The Astafactor ™ softgels will be formulated with *Haematococcus* algal meal produced with Aquasearch's proprietary technology. The algal meal will be processed into softgels and packaged into opaque plastic bottles and/or blister packs with state-of-the-art encapsulation and packaging equipment for nutraceutical and pharmaceutical applications. The product will be stabilized with an approved food-grade natural antioxidant.

The product will be marketed by:
Aquasearch, Inc.
73-4460 Queen Kaahumanu Hwy., #110,
Kailua-Kona, Hawaii 96740,
Tel: (808) 326 – 9301, Fax: (808) 326 – 9401

Information on the origin, manufacturing process and specifications, as well as proposed labelling and usage recommendations are attached in Section 4.

The recommended daily serving size is 5 mg astaxanthin.

000023

There has been already significant information made public to support the safety of astaxanthin and of *Haematococcus* as a dietary supplement (see Section 5 for details), including a New Dietary Ingredient Notification for *Haematococcus* algae, from Cyanotech Corporation, submitted to FDA on March 18, 1999. In addition, antioxidant properties of astaxanthin and its metabolic effects have been widely investigated (see section 6 for details) and results from these studies give no reason to believe that there should be any safety concern when astaxanthin is ingested at the recommended levels (see Section 5 for details). We feel however that our unique proprietary production technology and the well documented safety studies conducted on our product (Section 5), provide unique additional information to confirm that algal astaxanthin and more broadly *Haematococcus* produced with Aquasearch's technology is safe when used as an ingredient for dietary supplements according to our usage recommendations.

We contend that the algal meal used in these softgels is a natural product which is unadulterated and meets the evidence of safety under the Federal Food, Drug and Cosmetic Act se. 413 (350b)(a)(2). All other ingredients are approved ingredients for dietary supplements.

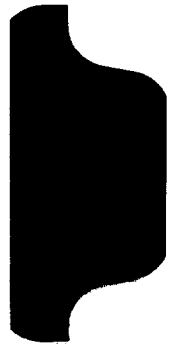
Furthermore the product is produced under strict quality control and according to current food GMP recommendations, with quality checks throughout manufacturing and after processing.

Thank you very much in advance for the attention that you will give to our notification. If you have any question, do not hesitate contacting us at (808)326-9301.

Sincerely yours,

Martin Guérin
V.P. Marketing & Sales
Aquasearch Inc.

000024



# DATASHEET

Aquasearch Inc.
bringing the oceans to life

## The AstaFactor™ softgels – 5 mg

Astaxanthin supplement

**DESCRIPTION AND USE:**

**The AstaFactor™ softgels (5 mg)** are a natural astaxanthin dietary supplement. Astaxanthin is one of nature's most common carotenoid pigments, found in animals such as salmon, krill, or lobster. It has very good biological antioxidant properties, with laboratory studies reporting up to 550 times higher antioxidant effect than vitamin E or up to 10 times higher than beta-carotene. *Haematococcus pluvialis*, a widely occurring micro-algae, is believed to be nature's richest source of astaxanthin. Aquasearch, Inc. has developed a unique proprietary closed photobioreactor and processing technology to ensure that the highest quality of astaxanthin is produced from *Haematococcus* under strict safety standards. The softgels are produced at state-of-the art encapsulation and packaging facilities. The safety of Aquasearch's astaxanthin product, The AstaFactor™ algal meal, the active ingredient in these softgels, has been confirmed in human clinical trials.*

*(* This statement has not been evaluated by FDA).*

This product is not intended to diagnose, treat, cure, or prevent any disease.

**DAILY SERVING SIZE:** Two softgels, equivalent to 5 mg astaxanthin.

**INGREDIENTS USED:**

Ingredients: Rice bran oil, algal meal (*Haematococcus pluvialis*), gelatin, glycerin, bee's wax, water, natural Vitamin E.

**SUPPLEMENT FACTS**

| | Amount per serving | Unit | % daily value |
|---|---|---|---|
| Total calories | 4 | cal | 0% |
| Total fat | 324 | mg | 5% |
| Sodium | 1 | mg | 0% |
| Total carbohydrate | 121 | mg | 0% |
| Protein | 176 | mg | 4% |
| Vitamin A | 48 | IU | 1% |
| Alpha tocopherol | 1 | mg | 9% |
| Total carotenoids* | 6 | mg | n.a.** |
| Total natural astaxanthin | 5 | mg | n.a.** |

* *Includes lutein and beta-carotene*

** *Not applicable*

**PHYSICAL CHARACTERISTICS**

| | |
|---|---|
| Soft gel capsule size: | approx. 382 mg |
| Soft gel capsule shape: | oval |
| Color: | Dark burgundy |
| Smell: | typical |

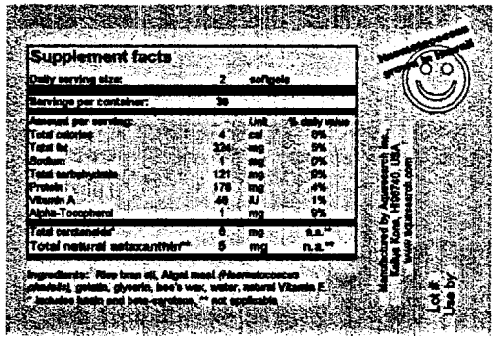
**PACKAGING:**

Opaque 75 cc plastic bottles or blister card packing.

000025



AQUASEARCH, INC., ©
73-4460 Queen Kaahumanu Hwy.,
Suite 110, Kailua-Kona, HI 96740, USA,

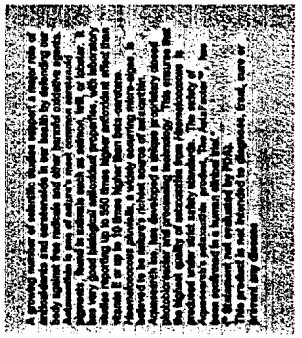



**Astaxanthin supplement**

TheAstaFactor™

Natural antioxidant for life

**60 softgels - 5 mg astaxanthin per serving**



1"6/4

6"2/8

000026

000027

## Supplement facts

| Daily serving size: | 2 | softgels | |
|---|---|---|---|
| Servings per container: | 30 | | |
| **Amount per serving:** | | **Unit** | **% daily value** |
| Total calories | 4 | cal | 0% |
| Total fat | 324 | mg | 5% |
| Sodium | 1 | mg | 0% |
| Total carbohydrate | 121 | mg | 0% |
| Protein | 176 | mg | 4% |
| Vitamin A | 46 | IU | 1% |
| Alpha-Tocopherol | 1 | mg | 9% |
| Total carotenoids* | 6 | mg | n.a.** |
| Total natural astaxanthin** | 5 | mg | n.a.** |

**Ingredients:** Rice bran oil, Algal meal *(Haematococcus pluvialis)*, gelatin, glycerin, bee's wax, water, natural Vitamin E.
* includes lutein and beta-carotene, ** not applicable

Haematococcus grown in Hawaii

Manufactured by Aquasearch Inc.,
Kailua Kona, HI96740, USA
www.aquasearch.com

Lot #:
Use by:

# Astaxanthin supplement

TheAstaFactor™

*Natural antioxidant for life*

**60 softgels - 5 mg astaxanthin per serving**

A growing number of scientific studies support a major role of antioxidants and carotenoids in our health by defending our body against free radicals and other harmful oxidative agents. Astaxanthin is one of nature's most common carotenoid pigments, found in animals such as salmon, krill, or lobster. It has very good biological antioxidant properties, with laboratory studies reporting up to 550 times higher antioxidant effect than vitamin E or up to 10 times higher than beta-carotene. *Haematococcus pluvialis*, a widely occurring micro-algae, is believed to be nature's richest source of astaxanthin. Aquasearch Inc. has developed a unique proprietary closed photobioreactor and processing technology. This ensures that the highest quality of astaxanthin from *Haematococcus* is produced under strict safety standards. The safety of Aquasearch's astaxanthin product, The AstaFactor™, has been confirmed in a human clinical trial.*
(* Statement not evaluated by FDA).
This product is not intended to diagnose, treat, cure or prevent any disease

6"2/8

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington, DC 20205


Aquasearch Inc.
Kona Production Facility
73-4460 Queen Kaahumanu Highway
Suite 110, Kailua-Kona, HI 96740 USA
Tel: (808)326-9301 Fax: (808)326-9401
WebSite: http://www.aquasearch.com/
E-mail: aqse@aquasearch.com

December 6, 1999

Re: New Dietary Supplement Notification for Astafactor ™, softgels, 5 mg. Stability studies

Dear Administrator,

As part of our New Dietary Supplement Notification for a new dietary supplement: The Astafactor ™, softgels, 5 mg, formulated with the new dietary ingredient *Haematococcus* algal meal, we have started stability studies (algal astaxanthin product in oil, and actual softgels) (see section 7) which will support the shelflife and "use by" recommendations that will appear on the label (see section 4).
Before the end of the 75 day period after notification we will send you an update on the progress of these studies.
At this stage, our preliminary studies show no or little degradation of astaxanthin when in oil at 40°C after 3 months. This should allow for a shelflife of at least 6 months at room temperature.

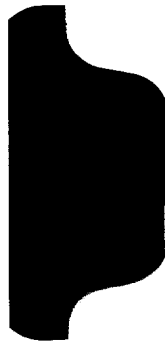
We plan to market The Astafactor ™, softgels, 5 mg, as a new human dietary supplements, 75 days after the acknowledgement of receipt of this notice, unless otherwise instructed by your agency.

Thank you very much in advance for the attention that you will give to our notification. If you have any question, do not hesitate contacting us at (808)326-9301.

Sincerely yours,

Martin Guérin
V.P. Marketing & Sales
Aquasearch Inc.

000023



Technical report

# *Haematococcus pluvialis* and astaxanthin safety for human consumption.

(TR.3005.001)


*Safety for human consumption of Haematococcus pluvialis algal meal and astaxanthin has been demonstrated by a number of studies:*

- *A recent 28-day rat study with Haematococcus pluvialis dry algal meal produced by Aquasearch's proprietary technology demonstrated that there was no observed sub-chronic toxicity at 50 mg/kg body weight, corresponding to 3,500 mg algal meal per 70-kg body weight of a typical adult man.*
- *No lethality was seen for Haematococcus pluvialis algae at doses up to 5000 mg/kg body weight, in an earlier, 13-day, single-dose (acute-toxicity), rat study.*
- *A human safety study demonstrated that daily ingestion of up to 1,140 mg Aquasearch's dry algal meal, for 29 days, did not result in any safety concern.*
- *A recent sub-chronic rat toxicity with Aquasearch's Haematococcus pluvialis algal meal showed no signs of toxicity after force-feeding rats up to 1.15 mg astaxanthin per kg body weight per day (equivalent to 80.5 mg astaxanthin per 70-kg body weight) for 28 consecutive days.*
- *In a human safety study with Aquasearch's algal astaxanthin, no sign of toxicity or safety concern was observed when volunteers ingested up to 19.25 mg astaxanthin per day for 29 days, while an earlier human study failed to find any harmful effect from 14.4 mg/day astaxanthin ingestion for two weeks.*
- *Pure astaxanthin (up to 80 mg/kg feed), is Generally Considered As Safe by FDA, for use in salmon diets. This can result in astaxanthin deposition of 10 to 15 mg/kg in salmon fillets. Levels of astaxanthin naturally occurring in seafood, and dietary studies on carotenoids, seafood, and salmon, also suggest that a daily serving of 5 mg astaxanthin, corresponding to 125 g of Sockeye salmon fillet or less than 100 g of krill, is safe.*
- *The proprietary technology and quality control developed by Aquasearch to produce Haematococcus pluvialis algal meal, ensure that the product meets dietary supplement safety standards.*

*CONCLUSION: a supplement containing 5 mg astaxanthin and 250 mg Aquasearch's Haematococcus pluvialis algal meal is safe for daily human consumption.*


Aquasearch Inc. ©, Revised: 12/06/99, 5:35 PM


*For further details, contact:*
Aquasearch Inc.,
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: (808)-326-9301, Fax: (808)-326-9401

Aquasearch's proprietary technology allows the production of a high quality algal meal containing 2% total astaxanthin or more. It is therefore a very good source of natural astaxanthin, a carotenoid pigment and biological antioxidant widely encountered in nature. Safety for human consumption of astaxanthin and *Haematococcus pluvialis* algae has been demonstrated by a number of studies.

**1. Toxicity studies**

**1.1. *Haematococcus* algae.**

**1.1.1. Human safety study**

In a recent clinical safety study with Aquasearch's *Haematocccus pluvialis* algal meal, 33 human volunteers (15 males and 18 females, age 28 to 62) ingested on a daily basis, for 29 consecutive days, either a Low Dose supplement containing 228 mg algal meal and 3.85 mg astaxanthin, or a High Dose supplement containing 1140 mg algal meal and 19.25 mg astaxanthin.[1] Volunteers underwent a complete medical examination before, during and at the end of the study. The physician, examined specifically, but not exclusively, the weight, skin coloration, general appearance, blood pressure, vision and eye, (near and distant vision, color vision, depth perception, eye condition), ears and nose, mouth, throat and teeth, chest and lungs, and reflexes, for each volunteer. This medical examination was complemented by extensive urine analyses and blood analyses (cell counts, hemoglobin, liver enzyme activity indicators, and other blood parameters) (Table 1). No ill effects or toxicity from ingestion of the supplement were observed, confirming the absence of toxicity of Aquasearch's *Haematococcus pluvialis* algal meal.

**1.1.2. Rat toxicity studies**

Absence of toxicity of *Haematococcus pluvialis* has also been demonstrated in rats and mice, widely accepted animal models for safety assessment of human dietary supplements.

A 28-day sub-chronic rat toxicity study, with *Haematococcus pluvialis* algal meal produced with Aquasearch's proprietary technology, failed to find any sign of toxicity of this algal meal.[2] Three groups of 20 rats each (10 males/10 females) were fed daily by gavage 0, 5, or 50 mg/kg algal meal in a corn oil suspension for 28 consecutive days (corresponding to daily doses of 0, 350 mg and 3,500 mg algal for 70-kg body weight). After sacrifice, the post-mortem observations, hematology and clinical chemistry failed to detect any sign of toxicity.

An earlier 13-day rat toxicity study demonstrated that the LD50 acute toxicity of *Haematococcus pluvialis* algal meal in rats was greater than 5000 mg/kg.[3] In this study, three separate groups of 10 Charles River CD rats (5 males and 5 females per group) were orally administered 5,000 mg/kg algal meal suspended in a 0.5% methylcellulose solution. Mortality, body weights, necropsy examination and pharmacotoxic signs were evaluated on each group. The study found no remarkable differences in body weights or visible abnormalities. The post-mortem examination after sacrificing the animals at the end of the study revealed no abnormalities.

Another acute toxicity trial was reported with male and female mice.[4] In this study, *Haematococcus pluvialis* algal meal was suspended in distilled water for gavage to give a 30% solution (w/v). The solution was forced in a single dose using a gastric probe, at dosages ranging from 10,417 to 18,000 mg/kg. No mortalities occurred and no abnormalities were observed in the post-mortem examination after sacrificing the animals. When converted to a 70-kg body weight, these doses are equivalent to single doses ranging from 729 g to 1,260 g.

**1.1.3. Other studies**

In salmonids, numerous experiments have shown that *Haematococcus pluvialis* could be incorporated in the diet at dosages ranging from 0.1% to 6% without any negative effect on growth or survival.[5,6,7,8] A recent report showed no indication of disease, toxicity or neoplasia in fish fed

*Haematococcus pluvialis* as a dietary source of astaxanthin.[4] The fish were reported in excellent nutritional status with abundant body fat. Studies have also indicated that feeding *Haematococcus pluvialis* can enhance growth and/or survival in trout and shrimp.[8-10]

**1.2. Astaxanthin.**

Astaxanthin naturally appears in the human diet when seafood such as salmon, red fishes, shrimp, krill or lobster are eaten.

**1.2.1. Human studies**

The recent clinical safety study, already mentioned above, proved the safety of astaxanthin from Aquasearch's *Haematocccus pluvialis* algal meal. [1] In that study, 33 human volunteers (15 males and 18 females, age 28 to 62) ingested on a daily basis, for 29 consecutive days, either 3.85 mg or 19.25 mg algal astaxanthin. As already mentioned earlier, extensive blood and urine analyses were conducted throughout the study (Table 1), and the physician conducted a detailed medical examination. Based on the results of these urine and blood analyses and the observations of the physician, no sign of toxicity from astaxanthin was detected even at the higher dose.

In a study with healthy human patients who ingested up to 14.4 mg/day astaxanthin for two weeks, no ill effect was reported.[11] On the contrary, a positive antioxidant effect of astaxanthin on serum Low Density Lipoprotein (LDL) was observed. In that study, thirteen healthy patients were selected, subdivided into 3 groups, and given three levels of astaxanthin daily, for two weeks, as follows: 5 patients fed 3.6 mg/day, 5 patients fed 7.2 mg/day, and 3 patients fed 14.4 mg/day. The astaxanthin was administered sublingualy in the form of a softgel capsule. Blood samples were taken and the LDL fraction was collected and exposed to the oxidising agent V-70, at 37°C. The study demonstrated that increasing doses of astaxanthin significantly and increasingly slowed down the oxidation of the LDL fraction.

**1.2.2. Rat toxicity studies**

In the recent study with Aquasearch's *Haematococcus pluvialis* algal meal, described above, rats ingested daily up to 1.15 mg astaxanthin per kg body weight (equivalent to 80.5 mg for 70-kg body weight per day), for 28 days, without showing any sub-chronic toxicity sign.

Other animal studies on the effects of astaxanthin have shown that even higher doses could be fed to rats for prolonged periods. Some of these studies have demonstrated beneficial results. In one study, feeding rats 500 ppm astaxanthin for 34 consecutive weeks resulted in reduced cancer occurrence in the intestinal and oral mucosa and improved the condition of the oral cavity.[12,13]

**1.2.3. Safety of astaxanthin in food salmon – safe daily dose of astaxanthin**

For years, astaxanthin has been added to aquaculture diets at levels of up to 200 mg/kg, without any toxic effect on target animals. Additionally, numerous studies have demonstrated improved growth, survival and immune response in fish and shrimp.[8-10,14-23] Astaxanthin is regularly added at 50 ppm or higher to commercial diets fed to food fish for prolonged periods, i. e., for up to 2 years in the case of farmed salmon. According to the Code of Federal Regulations, astaxanthin is Generally Recognized As Safe when used as a color additive in salmon foods, with a maximum inclusion of 80 mg/kg feed.[24] Numerous studies have shown that such an inclusion level results in accumulation of astaxanthin in the flesh of Atlantic salmon at levels between 4 and 10 mg/kg, and at even higher levels in other species (Table 2). These levels in Atlantic salmon are comparable to or slightly higher than levels observed in their wild counterparts, but lower than levels found in other wild salmon species found on the Pacific coast of the United States, where values as high as

58 ppm in Sockeye salmon were reported by a recent FDA study.[25] (Average of astaxanthin measurements in this study were 13.8 ppm in Coho salmon and 40.4 ppm in Sockeye salmon). It is interesting to note that the main astaxanthin isomer identified by the FDA researchers in the 5 species of wild Pacific salmon they studied, was the 3S,3'S stereo isomer, identical to that found in *Haematococcus pluvialis*.[8,25]

Salmon, a fish rich in omega-3 fatty acids, is considered a healthy food, and, like other sources of these poly-unsaturated fatty acids, is highly recommended by nutritionists.[26-29] According to an epidemiological study on Alaska's native and non-native residents, the lowest rate of ischaemic heart disease mortality, less than one-third that of US Caucasians, occurred in Alaskan Eskimos who lived in an area with documented patterns of high salmon consumption by individuals with high blood concentrations of omega-3 fatty acids.[28] Based on the salmon flesh astaxanthin values mentioned above, a daily consumption of a 200-g portion of wild Sockeye salmon with 40 ppm astaxanthin in the flesh would lead to a daily ingestion of 8-mg astaxanthin per day. From a different point of view, the intake of a 5-mg supplement of astaxanthin corresponds to eating 500 g per day of farmed rainbow trout or Atlantic salmon, 125 g of wild Sockeye salmon or less than 100 g of krill.

Based on these published data only, as well as the animal toxicity data publicly available, it may be inferred that the ingestion of 5 mg astaxanthin per day by an adult human is reasonably safe. This was further substantiated by Aquasearch's 29-day human safety study, which investigated the safety of 3.8 mg astaxanthin/day and 19 mg/day astaxanthin from *Haematococcus pluvialis* algal meal, i. e. almost four-fold higher than the assumed safe daily dose of 5 mg.[1] The results of the extensive blood and urine analyses and complete physical examinations before, during, and at the end of the trial period, raised no apparent safety concern. The data were reviewed by two independent physicians, a clinical pathologist and a professional pharmacotoxicologist, all of whom concurred that both doses were safe.

**2. Non-mutagenicity of *Haematococcus***

A recent study[30] reported no mutagenic effect of *Haematococcus pluvialis* algae, using a mutagenicity test with *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537, TA1538, and E.coli WP2 uvr A. In this experiment, *Haematococcus pluvialis* algal meal was formulated in a 50mg/mL solution of dimethyl sulfoxide. The formulation was spread onto petri dishes in the presence of the microbial cultures with positive controls. The positive controls (mutagenic agents): 2-(2-furyl)-3-5(5-nitro-2-furyl)acrylamide, 1-ethyl-2-nitro-3-nitrosoguanidine, 9-aminoacridine, 2-aminoanthracene, and 2-nitrofluorene, showed a remarkable increase in the number of reverent colonies in every case, compared to the solvent control.

**3. Carcinogenecity**

*Haematococcus pluvialis* is not known to have any carcinogenic effect or contain significant levels of recognized carcinogens. On the contrary, *Haematococcus pluvialis* contains a high level of astaxanthin which has widely demonstrated anticarcinogenic effects.[31-35]

**4. Heavy metals**

*Haematococcus pluvialis* algae produced and processed by Aquasearch for human food consumption meet the Federal Food and drug Administration's list of maximum tolerances:

| | |
|---|---|
| Heavy metals (as lead): | < 10.0 ppm |
| Mercury | < 1.0 ppm |
| Cadmium | < 0.5 ppm |

| | |
|---|---|
| Arsenic | < 2.0 ppm |
| Lead | < 5.0 ppm |

This has been confirmed by analyses of various batches (Lot HP980051[36] and Lot 990610Mix[37], a blend resulting from combining five batches: Lots 990513A, 990518B, 990520A, 990524A, 990526A, and therefore, highly representative of the quality of *Haematococcus pluvialis* algal meal produced with Aquasearch's technology).

**5. Bacteriology**

Manufacturing process follows FDA GMP recommendations for food supplements to avoid spoilage and contamination of *Haematococcus pluvialis* algal meal by harmful micro-organisms or other types of contaminants.

During the processing, the algal biomass is mechanically cell-broken to ensure a thorough rupture of cell walls, undergoes a pasteurisation process, and is dried to a moisture content less than 5%. The pasteurisation treatment ensures that the following bacteriological specifications in the final product are achieved, as confirmed by analyses by an independent laboratory[37]:

| | |
|---|---|
| Total aerobic plate count | <1,000 CFU |
| Total coliforms | <10/g |
| *E. coli* | <10/g |
| *Salmonella* | absence in 25 g |

**6. Other natural toxic compounds and toxicity risks**

Aquasearch is not aware of any significant or detectable levels of known carcinogenic or toxic compounds in *Haematococcus pluvialis* algae that could have a negative effect on human health. Analyses on the algae meal have demonstrated absence of Mycotoxins, and especially of aflatoxins.[36,37]

*Haematococcus pluvialis* may contain small amounts of canthaxanthin, a carotenoid pigment closely related to astaxanthin. Analyses have shown that cantaxanthin concentrations in *Haematococcus pluvialis* algal meal produced with Aquasearch proprietary technology could reach up to 2% of total astaxanthin concentration. In fact the proprietary technology developed by Aquasearch is aimed at maximising astaxanthin production: this minimises to its strict minimum the proportion of other carotenoids and intermediary metabolites in the algal meal. At the levels of canthaxanthin encountered in Aquasearch's algal meal, a daily dose of 5 mg algal astaxanthin as a supplement would entail also ingesting 0.1 mg canthaxanthin per day. Although canthaxanthin has been demonstrated to have positive metabolic effects such as an anticancer activity,[38] there has been reports that, at high doses for prolonged periods, it can have negative effects. One case of aplastic anemia associated with canthaxanthin ingested for tanning purposes, was reported a few years ago[39], while others[40] reported that, in some individuals who ingested up to 66 g cantaxanthin over 24 months (corresponding to an average daily ingestion of 90 mg cantaxanthin per day), for tanning purposes, crystalline formations started to appear in the retina. Those formations could potentially lead to functional impairments. It was however later demonstrated that these cantaxanthin deposits in the retina could be reversed.[39] Anyhow, the levels of canthaxanthin that would be ingested through a 5-mg astaxanthin dietary supplement formulated with Aquasearch's algal meal would be much lower than the doses which were observed to cause canthaxanthin maculopathy. Therefore, they should represent no safety risk. The rat toxicity and human safety study which were conducted with Aquasearch's algal meal confirmed this. It should also be noted that FDA has approved canthaxanthin as a color additive in fish foods (up to 80 mg/kg feed, which can result in canthaxanthin deposition levels of 4 to 12 mg/kg fillet) and broiler diets, as well as in

foods and drugs.[41] In foods, the limit authorized by FDA is 30-mg cantaxanthin per pound of solid food. The ingestion of 0.1-mg cantaxanthin in a dietary supplement containing 5 mg astaxanthin, is therefore well below the levels that would be encountered in foods that are considered safe by FDA.

**7. Product specifications**

A detailed description of the manufacturing process and of the specifications of *Haematococcus pluvialis* for use in dietary supplements are reviewed in a separate technical report.[42]

**8. Metabolic effects of astaxanthin**

Astaxanthin is a powerful natural antioxidant. There is a growing amount of scientific evidence not only on the safety of astaxanthin for human consumption, but on the positive metabolic effects that it may have. These findings have been reviewed in detail in Aquasearch Technical Reports TR.3002.001[43] and TR.3003.001 [44].

**9. Dietary studies - safe daily dose of algal astaxanthin**

Astaxanthin appears to be absorbed in the blood in the same way as other carotenoids. Carotenoids are absorbed by passive diffusion through the intestinal mucosa after being emulsified and solubilised in lipidic micelles which are incorporated into chylomicrons when exiting the intestinal mucosal cells.[45] They are transported in the blood after being transferred from the chylomicrons to lipoproteins. In a recent human study, a single dose of 100 mg dietary astaxanthin was not found to have any negative effect and demonstrated that astaxanthin has a similar absorption pattern to other carotenoids.[46] Astaxanthin was measured in the blood plasma of 3 middle-aged male subjects after ingestion of a single dose of 100 mg astaxanthin. Astaxanthin was readily absorbed and transported by various lipoproteins: chylomicrons/Very Low Density Lipoproteins, High Density Lipoproteins and Low Density Lipoproteins. Plasma levels of astaxanthin peaked at 1.2 mg/L (= 2 µmol/L) after 6 hours and progressively declined over the next 66 hours to a 0.2 mg/L level. These levels and duration are comparable to levels reported in the literature for other carotenoids.[47-49] Astaxanthin appears to be absorbed at a similar rate than beta-carotene which peaks in the serum after 6 to 9 h.[49] In mice, astaxanthin also appeared to be absorbed quite effectively, when compared to beta-carotene or lutein.[49]

The official recommended dietary intake for vitamin A is 1,000 retinol equivalents, for men, and 800 for women.[51] This corresponds to 6 mcg beta-carotene or 12 mcg of other pro-vitamin A carotenoids.[51] On the other hand, practical levels of carotenoid intake are significantly higher. Epidemiological studies in North Europe have found daily ingestion of carotenoids ranging from 2.9 to 7.6 mg/day,[52-54] while in the US, the level of carotenoids supplied by the "normal" diet is estimated to be 1.5 mg beta-carotene per day.[51] The Alliance for Aging Research, a US Citizen Advocacy organisation for research to improve the health and independence of older people, has recommended 10 to 30 mg beta-carotene per day for optimal health, and doses of 20 to 180 mg beta-carotene for many years have been used to treat erythropoietic protoporphyria, with not evidence of toxicity and without development of abnormally-elevated blood vitamin A levels.[51] In addition it should be noted that astaxanthin, unlike other carotenoids such as beta-carotene, has no provitamin A activity;[55,56] therefore it represents a lower risk of hyper-vitaminosis A.
It might be argued that because astaxanthin is closely related to canthaxanthin it could also have similar toxic effects as those described above. This argument is however invalidated by a number of facts:

- the proposed daily intake of astaxanthin (5-mg) is quite lower than the levels of canthaxanthin which were found to have toxic effects (up to 90 mg average daily intake for 24 months).

- In addition, the human safety study conducted with Aquasearch algal astaxanthin, found no changes in vision or eye condition in the patients. Another good indicator, skin coloration, did not change throughout the Aquasearch safety study.
- The post-mortem examination of the animals in Aquasearch's rat toxicity study also failed to find any adverse effect of astaxanthin supplementation at the doses tested.
- Researchers at the University of Illinois also reported that, in an animal model (rats), astaxanthin, unlike canthaxanthin, did not form crystalline depositions in the eye. [57] Furthermore, they demonstrated that astaxanthin can have a beneficial role in the protection of the eyes from UV-light damage.

In conclusion, based on published studies (reviewed above), on natural levels of astaxanthin found in seafood, and on the results of the studies conducted by Aquasearch, it appears that the consumption by a healthy adult of a daily dose of 5 mg astaxanthin, in the form of a supplement formulated with 250 mg (or less) *Haematococcus pluvialis* algal meal produced with Aquasearch's proprietary technology, represents no safety risk. This suggested dose is approximately four times lower than the high dose demonstrated as safe by Aquasearch's safety study.

**References**


1. Aquasearch Inc. 1999. A human safety trial of natural astaxanthin from Haematococcus pluvialis algae, produced with AQUASEARCH's proprietary technology. Confidential Report: RD.0100.001.
2. MB research laboratories. 1999. 28 day repeated dose oral toxicity study in rates. Confidential report to Aquasearch Inc.
3. International Research and Development Corporation. 1989. Acute oral toxicity Study in Rats for Microbio Resources Inc. Appendix 23, in: FDA website Docket Number 95S-0316, Report #45, (URL: http://www.fda.gov/ohrms/dockets /dailys/062199/rpt00045.htm). Access date: November 27, 1999.
4. Koyo Mercantile Company ltd. 1988. Acute Toxicity Test for Riken Vitamin K.K. Appendix 24, in FDA website, Docket Number 95S-0316, Report #45, (URL: http://www.fda.gov/ohrms/dockets /dailys/062199/rpt00045.htm). Access date: November 27, 1999.
5. Spitsbergen J. 1997. Gross pathologic examination of salmonids from dietary study with Natural Astaxanthin (*Haematococcus* algae meal). Appendix 26, in FDA website Docket Number 95S-0316, Report #45, (URL: http://www.fda.gov/ohrms/dockets /dailys/062199/rpt00045.htm). Access date: November 27, 1999.
6. Sommer T.R., F.M.L.D. Souza and N.M. Morissy. 1991. Pigmentation of adult rainbow trout, Onchorhynchus mykiss, using the green alga *Haematococcus pluvialis*. Aquaculture, 106, 63-74.
7. Choubert G. and O. Heinrich. 1993. Carotenoid pigments of the green *alga Haematococcus pluvialis* : assay on rainbow trout *Onchorhynchus mykiss*, pigmentation in comparison with synthetic astaxanthin and cantaxanthin. Aquaculture 112, 217-226.
8. Aquasearch Inc. 1999. Technical report TR2102.001: Aquaxan™ HD algae meal use in aquaculture diets: Enhancing nutritional performance and pigmentation.
9. Darachai J., S. Piyatiratitivorakul, P. Kittakoop, C. Nitithamyong, P. Menasveta. 1998. Effects of astaxanthin on larval growth and survival of the giant tiger prawn, Penaeus monodon. the Vth Asian Fisheries Forum in Chiang Mai, Thailand (November 11-13, 1998).

10. Christiansen R., O. Lie, O.J. Torissen. 1995. Growth and survival of Atlantic *salmon Salmo salar* L. fed different dietary levels of astaxanthin. First-feeding fry. Aquac., Nutr. 1, 189-198.
11. Miki, W., K. Hosada, K. Kondo, and H. Itakura. 1998. Astaxanthin-containing drink. Japanese Patent #10155459 [in Japanese].
12. Tanaka, T., T. Kawamori, M. Ohnishi, H. Makita, H. Mori, K. Satoh, and A. Hara. 1995a. Suppression of azomethane-induced rat colon carcinogenesis by dietary administration of naturally occurring xanthophylls astaxanthin and canthaxanthin during the postinitiation phase. Carcinogenesis 16: 2957-2963.
13. Tanaka, T., H. Makita, M. Ohnishi, H. Mori, K. Satoh, and A. Hara. 1995b. Chemoprevention of rat oral carcinogenesis by naturally occurring xanthophylls, astaxanthin and canthaxanthin. Cancer Res. 55: 4059-4064.
14. Christiansen R., O. Lie, and O.J. Torissen. 1994. Effect of astaxanthin and vitamin A on growth and survival of Atlantic salmon fry, *Salmo salar* L. Aquac., Fish. Manag. 25:903-914.
15. Verlhac V., J. Gabaudan, and J. Schierle. 1995. In-vitro anti-oxidant properties of astaxanthin on rainbow trout immune cells. In: Developmental and comparative immunology. Clem, L.W. Warr G.W. (Eds.). The VIth ISDC congress. Abstracts. P889. Presented at the Nordic Symposium on Fish Immunology. May 1995, Reykiavik, Iceland.
16. Verlhac V., Gabaudan J., Schierle J. 1995. Influence of astaxanthin on non-specific immune response of rainbow trout. Presented at the Nordic Symposium on Fish Immunology. May 1995, Reykiavik, Iceland. Clem, L.W. Warr G.W. (Eds.). The VIth ISDC congress. Abstracts. Presented at the Nordic Symposium on Fish Immunology. May 1995, Reykiavik, Iceland.
17. Chien Y.H., S.C. Jeng. 1992. Pigmentation of Kuruma prawn, *Penaeus japonicus* Bates, by various pigment sources and levels and feeding regimes. Aquaculture, 102, 333-346.
18. Tanaka Y., H. Matsuguchi, T. Katayama, K.L. Simpson, C.O. Chichester. 1976. The biosynthesis of astaxanthin. XVIII. The metabolism of the carotenoids in the prawn, *Penaeus japonicus* Bates. Bull. Jpn. Soc. Sci. Fish., 42:197-202.
19. Menasveta P. 1995. Role of micro-nutrients in increasing disease resistance in shrimp. 2nd. Roche Aquaculture Centre Conference on Shrimp Nutrition and Disease. June 15, 1995, Bangkok, Thailand, Ed. K. Kurmali.
20. Nègre-Sadargues G., R. Castillo, H. Petit, S. Sance, R.G. Martinez, J-C.G. Choubert, J-P. Trilles. 1993. Utilisation of synthetic carotenoids by the prawn *Penaus japonicus* reared under laboratory conditions. Aquaculture 110, 151-159.
21. Thongrod S., A. Tansutapanich, O.J. Torissen. 1995. Effect of dietary astaxanthin and supplementation on accumulation, survival, and growth in post-larvae of *Penaeus monodon* Fabricius. In P.Lavens, E. Jaspers and I. Roelants (eds.). Larvi'95 – Fish & Shellfish Larviculture Symposium. European Aquaculture Society, Special Publication, No. 24, Gent, Belgium, 251-254.
22. Kurmali K. 1995. Shrimp Nutrition and diseae: role of vitamins and astaxanthin. 2nd. Roche Aquaculture Centre Conference on Shrimp Nutrition and Disease. June 15, 1995, Bangkok, Thailand. Ed. K. Kurmali.
23. Chien Y.H. 1996. Biological effects of astaxanthin in shrimp, a review. 3rd Roche conference on nutrition and disease. December 12, 1996, Bangkok, Thailand, Ed. B. Hunter, 73-81.
24. Code of Federal Regulations, Title 21, Volume 1, Parts 1 to 99, Revised as of April 1, 1999, via GPO Access[CITE: 21CFR73], Page 332-377, Part 73: Listing of color additives exempt from certification, Subpart A: Foods, Section 73-35: astaxanthin, Access date: November 27, 1999. URL: http://frwebgate5.access.gpo.gov/cgi-bin/waisgate.cgi?WAISdocID=4096121134+2+0+0&WAISaction=retrieve

25. Turujman S.A., W. G. Wamer, R.R. Wei, R.H. Albert. 1997. Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. J. AOAC Int., 80(3): 622-632.
26. Kromhout D., E.J. Feskens, C.H. 1995. The protective effect of a small amount of fish on coronary heart disease mortality in an elderly population. Int. J. Epidemiol. Apr;24(2):340-5.
27. Keli S.O., E.J. Feskens, D. Kromhout. 1994. Fish consumption and risk of stroke. The Zutphen Study. Stroke, 25(2):328-32.
28. Davidson M., L.R. Bulkow, B.G. Gellin. 1993. Cardiac mortality in Alaska's indigenous and non-Native residents. Int. J. Epidemiol.;22(1):62-71.
29. Horrocks L.A., Y.K. Yeo. 1999. Health benefits of docosahexaenoic acid. Pharmacol. Res.;40(3):211-25.
30. Cyanotech Corp. 1999. Haematococcus algae safety summary. FDA website Docket Number 95S-0316, Report #45, (URL: http://www.fda.gov/ohrms/dockets /dailys/062199/rpt00045.pdf)062199.htm). Access date: November 27, 1999.
31. Gradelet, S., P. Astorg, J. LeClerc, J. Chevalier, M.-F. Vernevaut, and M.-H. Siess. 1996. Effects of canthaxanthin, astaxanthin, lycopene and lutein on liver xenobiotic-metabolizing enzymes in the rat. Xenobiotica 26: 49-63.
32. Jyonouchi, H., R.J. Hill, Y. Tomita, and R. A. Good. 1991. Studies of immunomodulating actions of carotenoids. I. Effects of beta-carotene and astaxanthin on murine lymphocyte functions and cell surface marker expression in in vitro culture system. Nutr. Cancer 16: 93-105.
33. Jyonouchi, H., L. Zhang, and Y. Tomita. 1993. Studies of immunomodulating actions of carotenoids. II. Astaxanthin enhances in vitro antibody production to T-dependent antigens without facilitating polyclonal B-cell activation. Nutr. Cancer 19: 269-280.
34. Jyonouchi, H., L. Zhang, M. Gross, and Y. Tomita. 1994. Immunomodulating actions of carotenoids: Enhancement of in vivo and in vitro antibody production to T-dependent antigens. Nutr. Cancer 21: 47-58.
35. Tomita, Y., H. Jyonouchi, R.W. Engelman, N.K. Day, and R.A. Good. 1993. Preventive action of carotenoids on the development of lymphadenopathy and proteinuria in MRL-lpr/lpr mice. Autoimmunity 16: 95-102.
36. Aspland and James Ltd. 1998. Certificate of analysis. Algal meal Lot # HP980051.
37. Warren Analytical laboratory. 1999. Laboratory Analysis Report on Lot # 990610 Mix *H.pluvialis* flakes. Confidential report to Aquasearch Inc.
38. Gerster H. 1993. Anticarcinogenic effect of common carotenoids. Int. J. Vitam. Nutr. Res., 63(2):93-121.
39. Leyon H., A.M. Ros, S. Nyberg, P. Algvere. 1990. Reversibility of cantaxanthin deposits within the retina. Acta. Ophthalmol. (Copenh.), 68(5):607-611.
40. Boudreault G., P. Cortin, L.A. Corriveau, A.P. Rousseau, Y. Tardif, M. Malenfant. 1983. Canthaxanthine retinopathy: 1. Clinical study in 51 consumers. Can. J. Opthalmol., 18(7):325-328.
41. U.S. F.D.A. 1999. Section 73.75: Canthaxanthin. Code of Federal Regulations, 21(1)73: 337-338.
42. Aquasearch Inc. 1999. Technical report TR3004.001. Haematococcus pluvialis algal meal produced with Aquasearch's proprietary technology: a unique source of natural astaxanthin and algal nutrients.
43. Aquasearch Inc. 1999. Technical report TR.3002.001. Astaxanthin as an antioxidant: a summary.
44. Aquasearch Inc. 1999. Technical report TR.3003.001. Astaxanthin and health: a summary.

45. Furr H. C. and R.M. Clark. 1997. Intestinal absorption and tissue distribution of carotenoids. Nutritional Biochem. 8:364-377.
46. Osterlie M., B. Bjerkeng, S. Liaaen-Jensen. 1999. Pigments in food technology, Proc, 1st. Int. Congr. PFT., March 24-26, Sevilla, Spain.
47. Kostic D., W.S. White, J.A. Olson. 1995. Intestinal absorption, serum clearance, and interactions between lutein and beta-carotene when administered to human adults in separate or combined oral doses. Am. J. Clin. Nutr., 62: 604-610.
48. Clark R.M., L. Yao, L. She, H.C. Furr. 1998. A comparison of lycopene and canthaxanthin absorption: using the rat to study the absorption of non-provitamin A carotenoids. Lipids, 33(2):159-63.
49. Gärtner C., W. Stahl, et al. 1996. Preferential increase in chylomicron levels of the xantophylls lutein and zeaxanthin compared to beta-carotene in the human. Int. J. for Vit. and Nutr. Res., 66:119-125.
50. Park JS, BP Chew, TS Wong, JX Zhang, NS Magnuson. 1999. Dietary lutein but not astaxanthin or beta-carotene increases pim-1 gene expression in murine lymphocytes. Nutr. Cancer 33(2):206-212.
51. VERIS. 1996. Carotenoids – Fact Book. Ed. VERIS, La Grange, IL., USA, 32pp.
52. Golbohm R.A., H.A. Brants, K.F. Hulshof, PA van den Brandt. 1998. The contribution of various foods to intake of vitamin A and carotenoids in the Netherlands. Int. J. Vitam. Nutr. Res. 68(6):373-383.
53. Heinonen M. 1991. Food groups as sources of retinoids, carotenoids and Vitamin A in Finland. Internat. J. Vit. Nutr. Res. 61:3-9.
54. Faure H., V. Fayol, C. Galabert, P. Grolier, GL Moel, J. Stephens, F. Nabet. 1999. Carotenoids: 2. Diseases and supplementation studies. Ann Biol. Clin. (Paris), 57(3):273-282.
55. Jyonouchi, H., S. Sun, and M. Gross. 1995. Effect of carotenoids on in vitro immunoglobulin production by human peripheral blood mononuclear cells: Astaxanthin, a carotenoid without vitamin A activity, enhances in vitro immunoglobulin production in response to a T-dependent stimulant and antigen. Nutr. Cancer 23:171-183.
56. Jyonouchi, H., S. Sun, Y. Tomita, and M.D. Gross. 1995. Astaxanthin, a carotenoid without vitamin A activity, augments antibody responses in cultures including T-helper cell clones and sub-optimal doses of antigen. J. Nutr. 124:2483-2492.
57. Tso, M.O.M., and T.-T. Lam. 1996. Method of retarding and ameliorating central nervous system and eye damage. U.S. Patent #5527533.
58. Goralczyck R., S. Buser, J. bausch, W. Bee, U. Zuhlke, F.M. Barker. 1997. Occurrence of birefringent retinal inclusions in cynomolgus monkeys after high doses of cantaxanthin. Invest. Ophthalmol. Vis. Sci., 38(3):741-752.
59. Leyon H., A.M. Ros, S. Nyberg, P. Algvere. 1990. Reversibility of cantaxanthin deposits within the retina. Acta Ophthalmol. (Copenh.), 68(5):607-611.
60. Aquasearch Inc. 1999. Technical report TR.3002.001. Astaxanthin as an antioxidant: a summary.
61. Aquasearch Inc. 1999. Technical report TR.3003.001. Astaxanthin and health: a summary.

**Table 1: List of analyses in human safety study conducted on Aquasearch's *Haematococcus pluvialis* algal meal.[1]**

| | |
|---|---|
| **Blood chemistry analyses** | |
| Serum glutamate pyruvate transaminase (SGPT) | |
| Lactate dehydrogenase (LDH) | |
| Glucose | |
| Total protein | |
| Total bilirubin | |
| Blood urea nitrogen (BUN) | |
| Creatinine | |
| Total cholesterol | |
| High-density lipoprotein (HDL) cholesterol | |
| Triglycerides | |
| Low-density lipoprotein (LDL) cholesterol (calculated) | |
| Albumin | |
| Globulin | |
| **Complete blood count** (CBC) | |
| White blood count (WBC) | |
| Red blood count (RBC) | |
| Hemoglobine (HGB) | |
| Hematocrit (HCT) | |
| Mean corpuscular volume (MCV) | |
| Mean corpuscular hemoglobin (MCH) | |
| Mean corpuscular hemoglobin concentration (MCHC) | |
| Red cell distribution width (RDW) | |
| Platelet count | |
| Neutrophil (segs) | |
| Lymphocytes | |
| Monocytes | |
| Eosinophils | |
| Bsophils | |
| Red blood cell morphology | |
| Coagulation test (activated partial thromboplastin time, PTT) | |
| **Urinalysis tests** | |
| Color | pH |
| Appearance | Protein |
| Specific gravity | Glucose |
| Leukocyte esterase | Ketones |
| Nitrite | Urobilinogen |
| Blood | Bilirubin |

**Table 2. Levels of of astaxanthin in selected types of seafoods[8]**

| Seafood type | Astaxanthin Content (mg/kg) | Free/esterified | Main isomer |
|---|---|---|---|
| Sockeye salmon | 26-37 | Free,esterified** | 3*S*,3'*S* |
| Coho salmon | 9-21 | Free,esterified** | 3*S*,3'*S* |
| Chum salmon | 3-8 | Free,esterified** | 3*S*,3'*S* |
| Chinook salmon | 8-9 | Free,esterified** | 3*S*,3'*S* |
| Pink salmon | 4-6 | Free,esterified** | 3*S*,3'*S* |
| Atlantic salmon | 3-11 | Free,esterified** | 3*S*,3'*S* |
| Rainbow trout | 1-3 | Free,esterified** | 3*S*,3'*S* |
| salmon eggs | 0-14 | Esterified*** | N.A. |
| Red seabream | 2-14 | Esterified*** | N.A. |
| Red seabream eggs | 3-8 | N.A. | N.A. |
| *Peneaus monodon* | 10-150 | Esterified,free** | 3*S*,3'*S* |
| Lobster | | Esterified,free** | N.A.* |
| Krill | 46-130 | Esterified*** | 3*R*,3'*R* |
| Krill oil | 727 | Esterified*** | 3*R*,3'*R* |
| Crayfish meal | 137 | Esterified*** | N.A.* |
| Artic shrimp | 1160 | Esterified*** | 3*S*,3'*S* |
| *Haematococcus pluvialis* | 10,000-30,000 | Esterified*** | 3*S*,3'*S* |

* Most crustaceans studied appear to have mostly the 3S-3'S form, unlike Krill.
** depending on tissues, free or esterified astaxanthin may be found
*** also contain a small proportion of free astaxanthin

Aquasearch Inc.
bringing the oceans to life

# *A human safety trial of natural astaxanthin from Haematococcus pluvialis algae, produced with AQUASEARCH's proprietary technology.*

## REPORT: RD.0100.001

## Date: December 6, 1999

THIS DOCUMENT CONTAINS CONFIDENTIAL INFORMATION PROPRIETARY TO AQUASEARCH THAT MAY BE DIVULGED ONLY TO AUTHORIZED PERSONS. NO COPY ALLOWED WITHOUT AQUASEARCH PRIOR WRITTEN CONSENT.
ISSUE #: 001

Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401
Confidential

# Table of contents





Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401
Confidential



000042

## INTRODUCTION

- The purpose of this study was to evaluate the human tolerance of **astaxanthin from a natural source** (the freshwater alga *Haematococcus pluvialis*) produced with Aquasearch's proprietary technology and see what effects (good and bad) it may have on the health of human volunteers. The results of the study were intended to be included in the submission to regulatory authorities for marketing approval of:
  - *Haematocccus pluvialis* algae meal produced with Aquasearch's proprietary technology as a source of astaxanthin and as an ingredient in dietary supplements,
  - Dietary supplements formulated with *Haematococcus pluvialis* produced with Aquasearch's technology.
- This research was done because astaxanthin is known from *in vitro* tests and from animal model studies to have powerful biological antioxidant properties, and has not been found to have any indication of risks to humans.

## EXPERIMENTAL DESIGN

### Clinical study

This study was conducted under the supervision of Clinical Laboratories of Hawaii (CLH), who were responsible for conducting all veni-punctures and analyses, and two independent medical practitioners responsible for the medical examinations of the volunteers (one in Honolulu, Hawaii, Dr. M. Kuge, M.D., for volunteers in that location, and one in Kailua-Kona, Hawaii, Dr. A. Silver, M.D. for those volunteers located there). Each volunteer was given an informed consent form to review and sign, informing them of the objective, the conditions, and potential risks of the study (Appendix B). Volunteers were being given sufficient time for consideration and had the possibility, if they chose, of dropping out of the study at any time. In order to be selected for the study, volunteers had to be an adult (over 18 years of age), in good health, and not regularly taking any medications nor any dietary supplements that were high in carotenoids (i. e., 4 mg/day or more of beta-carotene or other carotenoids) or in vitamin E (i. e., 400 IU vitamin E daily or more).

A minimum number of 30 volunteers, with as equal as possible number of male and female subjects, was judged appropriate after consultation with a professional pharmacologist.

Volunteers were randomly divided in two groups of subjects. The first group was asked to consume a target astaxanthin dose of 5 mg per day for four weeks, the LOW dose, while the second was asked to consume a target astaxanthin dose of 25 mg per day for four weeks, the HIGH dose.

Prior to the start of the study, volunteers were asked to report for a complete physical examination by one of the two physicians, and for blood and urine sampling for subsequent standard clinical analyses (blood and urine tests). The medical examination followed a standard


Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

Confidential

template of CLH, and included, among other observations, the health history, weight, skin coloration, general appearance, blood pressure, vision and eye, (near and distant vision, color vision, depth perception, eye condition), ears and nose, mouth, throat and teeth, chest and lungs, reflexes, etc...(see Appendix C for standard template and report). This first physical examination and first set of clinical analyses (**T1**) was used to determine if each volunteer was eligible for this study. Volunteers were considered eligible for the full study if the results of the examination and clinical chemistry analyses fell within ranges accepted as "normal" (i. e., within CLH standard "normal range" established from the laboratory's historic data, and considered representative of a normally healthy population) (Table 1). The responsibility for this decision of retaining or rejecting any volunteer was left to an experienced pathologist at CLH. Because of the inherent variability of the analytical methods used by CLH and of the parameters within a healthy population, some volunteers with a few values marginally outside of the reference range were maintained in the study, provided the general profile of the results demonstrated that their general health status could be considered "normal".

Approximately one week later, the blood and urine testing were repeated. For each patient, the results of this **second set of clinical analyses (T2)**, combined with the first set, were intended to provide a clinical chemistry baseline for this study.

Three to seven days after the start of the study (i. e., three to seven days after the first consumption of the astaxanthin tablets), patients underwent a **second physical examination** and **third set of clinical analyses (T3)** (blood and urine tests).

At the end of the study (i. e., on the 30th day after the first consumption of the astaxanthin tablets), volunteers underwent a **third physical examination** and a **fourth set of clinical analyses** (blood and urine tests) **(T4)**.

Due to limited time availability of physicians and volunteers, dates of medical examinations as well as dates of initial and intermediate sampling varied slightly from one volunteer to the other. This was deemed acceptable since the health status of each individual volunteer was considered independent from that of the other volunteers.

Any deviation from the baseline defined from the two first sets of analyses and not within the "normal" range values used as standard control values by CLH in their routine analysis reports (see appendix 3) was recorded and subject to review by the physicians and CLH pathologists, to determine whether it represented any safety concerns.

**Test and procedures**

Tests and procedures conducted in this study by CLH and the physicians were routine procedures that are usually done as part of a complete physical examination. None of these procedures were experimental. The standard physical examination form used by CLH was used as a guide in the



Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401



Confidential

physical examination (see Appendix C), and was complemented by questions and notes based on each physician's own regular practice. This medical examination included, among other observations, the weight, skin coloration, general appearance, blood pressure, vision and eye, (near and distant vision, color vision, depth perception, eye condition), ears and nose, mouth, throat and teeth, chest and lungs, reflexes, etc. A copy of the first set of analyses was given to each volunteer.

- **Clinical samples.** Blood and urine samples were taken four times during the research study as described above. Volunteers were advised to fast for 12 hours (minimum) after a regular dinner the night before the sampling, and to avoid alcohol ingestion 24 hours before specimen collection. Only water was allowed after dinner. Samples were obtained in the morning. Blood was drawn using CLH routine venipuncture procedures. Each sample collection included one 10 mL serum separator tube (SST) and one 4.5 mL sodium citrate tube (for blood analyses), and one 5-10 mL urine sample.

- The following blood tests were performed at each blood sampling, according to CLH standard operating procedures:
  - **Blood chemistry analyses,** included: serum glutamate pyruvate transaminase (SGPT or ALT), glucose, lactate dehydrogenase (LDH), total protein, total bilirubin, blood urea nitrogen (BUN), creatinine, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol (calculated), albumin, and globulin (detailed procedures described in CLH Hitachi 747 Methodology manual).
  - **A complete blood count** (CBC) was performed, including white blood count (WBC), red blood count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count, neutrophil (segs), lymphocytes, monocytes, eosinophils, basophils, and red blood cell morphology. Finally, a **coagulation test** (activated partial thromboplastin time, PTT) was performed. (Detailed procedures described in CLH standard operating procedures manual).

- The **urinalysis tests** included color, appearance, specific gravity, leukocyte esterase, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, and blood. (Detailed procedures described in CLH standard operating procedures manual).

**Test material**

A detailed chemical analysis and bacterial analysis of the Lot #990610Mix of *Haematococcus pluvialis* algal meal used for the testing had been performed by independent laboratories to confirm nutrient content, low bacterial counts and absence of heavy metals or aflatoxins (Warren Analytical Laboratory, Laboratory Analysis Report, 1999, Work orders No. 9914382, 9914380, 9914389, 9914353). Astaxanthin content of the algal meal assayed externally (Akvaforsk, 1999, Analysis report, Project S827) was confirmed by Aquasearch internal analysis (Aquasearch, 08/05/99, Certificate of Analysis, Lot 990610Mix). The test product was considered safe and


Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

Confidential

representative of the intended future commercial product to be used in dietary supplements. The algal meal used for this study was produced and processed at Aquasearch's commercial production facilities in Kona, according to the process described in Aquasearch's Technical Report TR.3004.001. The specific lot tested was produced by mixing 5 individual production batches. It was then packed in aluminum-lined airtight heat-sealed bags, stored at –20 °C to prevent any loss in astaxanthin content, and then sent to a commercial manufacturer of dietary supplements with commercial tabletting and bottling facilities. There the algal meal was processed into a compressed hard tablet and bottled in air-tight, tamper-proof opaque plastic bottles. The proprietary formulation (Appendix A) contained only the algal meal, widely accepted food-grade antioxidants and inactive excipients, destined to ensure adequate product stability and physical appearance. Volunteers were asked to ingest either one or five tablets daily for a period of 28 days (due to scheduling issues for the last venipuncture, the period was extended to 29 days). The supplement tablets were tested for aerobic plate count prior to the start of the study to confirm absence of recontamination during the tabletting and bottling phase. A verification of the astaxanthin content in each tablet was conducted by Aquasearch's standard spectrophotometric analysis, as described in Aquasearch's Technical Report TR.1002.001, at the end of the study to confirm the amount of astaxanthin ingested.

**Analysis and interpretation of results.**

To determine whether the product could be considered safe, results were looked at in several ways:

1) A qualitative assessment was conducted volunteer by volunteer. Volunteers were grouped into two categories:

- Those volunteers for which the final analytical results remained within CLH "normal range" of values, or within the individual baseline range determined by the first two sets of analyses (T1 and T2), were considered as normal.
- Those for which the final result went out of the CLH "normal range" of values, and outside of the initial baseline, were further discussed in detail with CLH's pathologist and the physician who had examined them, to interpret the deviation and define whether this raised any safety concern.

2) A quantitative assessment to determine whether the supplement had an effect, whether negative or positive, was also conducted, by running a two-way ANOVA, with proportional replication, to compare T3 v.s. the baseline (T1 & T2) and T4 v.s. the baseline (T1 & T2), for each parameters, at each dose. Whenever a significant difference from the baseline was detected ($P<0.05$) for one parameter at a specific dose and sampling time (T3 or T4), it was compared with the three other comparisons for this specific parameter, to determine if the change could be attributed to the supplement and represented any safety concern (Zar, 1984: "Biostatistical Analysis", Prentice-Hall, Inc., Englewood Cliffs, New Jersey).



Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401



Confidential

## RESULTS

Summary of analyses results are presented in Table 3 (Low dose) and Table 4 (high dose) . Details of the statistical evaluation are in Appendix D

**Product analyses**

Aerobic plate count of the tablets remained <1000 CFU/g, confirming that there had been no or little contamination during the tabletting process.

Astaxanthin content in the tablets was measured a few days after the end of the study. The actual average total astaxanthin content in each tablet was 3.85 mg at the end of the study, compared to a targeted 5 mg. This is believed to have resulted from losses during tabletting and storage.

**Volunteer participation and profile**

From the initial 33 volunteers, all were retained despite a few values outside of the "normal range" measured at the first sampling, but still considered as acceptable (Table 1). Fifteen volunteers were examined in Honolulu and 18 in Kona. Sex ratio consisted of 15 men and 18 women (5 men in Honolulu, 10 men in Kona, 10 women in Honolulu, 8 women in Kona). No volunteer dropped out of the study or missed a blood drawing or physical examination. All volunteers had their blood and urine samples taken on the same day at the end of the study, except for one who had the sample taken the following day.

Age ranged from 27 to 62, with an average age of 41.5 years.

Out of a theroretical total of 5808 analyses from the 4 samplings, 44 types of analyses and 33 volunteers, only 16 of those expected values (= 0.28%) were missing in CLH's reports, purportedly as a result of lost or discarded samples, of unperformed analysis, or of aberrant result.

**Qualitative evaluation of analyses results**

At the end of the study, at the fourth sample collection, out of 1,444 analytical results, only 78 of the analysis results (5.4% of the total number of analyses at sampling #4), were reported abnormal by CLH. Out of these 78 "abnormal" results, only 19 (or 1.3% of the total at sampling #4) represented a change from an initial baseline within the "normal range" to a final value outside of the "normal" range. Volunteers for which this change occurred were reviewed individually and none of those changes was considered a result of the supplement or raised a safety concern, as is summarized below:

**Volunteer 4 (female):** traces of blood appeared in the urine. This was believed by the physician to be most likely associated with menstruation, and not a result of the ingestion of the supplement. It was considered a benign symptom.

**Volunteer 6 (female):** an increase in lymphocytes and decrease in neutrophils was considered as unspecific by the physician, and most probably a result of a minor viral infection.

**Volunteer 12 (female):** an increase in monocytes and blood triglycerides was noted and not judged to be of concern or attributable to the supplement. The change in the first parameter could have resulted from a viral infection, and the change in the second was believed to be dietary.



Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401



**Confidential**

**Volunteer 17 (male):** Glucose level of 112 was just outside of the normal range (110) and considered not unusual by the physician and pathologist.
**Volunteer 20 (male):** Had a drop in haemoglobin (13.9) just below "normal range" (14.0), which was still considered normal by the physician, and within laboratory variability.
**Volunteer 21 (female):** had ketones in the urine, which the physician described as benign and common when patients have been fasting.
**Volunteer 22 (female):** had a minor increase in neutrophils (71) just above "normal range" (70), within laboratory analytical variability, and some traces of blood in urine most likely associated with menstruation.
**Volunteer 27 (female):** had a small drop in RBC and haemoglobin, and an increase in platelets. The physician judged this benign and most likely associated with menstruation.
**Volunteer 29 (female):** had a small drop in cholesterol/HDL ratio to just below the minimum of the "normal range" which was considered favorable and raised no safety concern.
**Volunteer 32 (female):** experienced an increase in ALT (SGPT) (33) to just above the normal range (30) which was considered of no concern and non-specific. The small drop in urine specific gravity (1.002) to just below minimum Normal Value (1.005) was also without significance.
None of those changes constituted a pattern that was deemed to be of any concern or could be attributed to the ingestion of the supplement.

**Qualitative evaluation of medical examinations**
Both physicians reported no sign of any toxicity associated with the ingestion of either the low or the high dose. The most common observation was that a number of patients noticed a reddening of the stools, indicating that part of the algae may have remained undigested. Others indicated a possible loosening of the bowel movements, although diarrhea was not reported. There was no notice of any change in skin pigmentation that could be attributed to the supplement. Neither the vision, nor the condition of the eyes of the volunteers was deemed affected by the ingestion of the supplement. All volunteers remained basically healthy and sound throughout the study with a few experiencing minor changes in condition not associated to the supplement, such as small injuries, colds, etc...

**Statistical evaluation of analytical results on effect of astaxanthin supplementation with low and high astaxanthin dose.**
Statistical results have been summarized in Appendix D. Average values all remained within CLH's "normal" range. Some statistically significant changes were observed throughout the study and have been reviewed individually (Appendix D), although none raised any safety concern. Most changes could not be attributed to the supplement since no clear pattern or confirmation from one dose to the other or from one sampling to the other, could be identified. Those changes have been judged minor and of no safety consequence by both physicians and the pathologists, and are most likely deriving from other factors than the supplementation, such as unidentified factors within the population samples, the natural variability and sensitivity of the analytical methods, the natural variability of those parameters, or the power of the statistical test


Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

Confidential

used. A good illustration is the small significant drop in BUN which occurred only in the population taking the low dose, but not in the volunteers ingesting the high dose.

**Conclusion**

Neither the CLH pathologists, nor the two physicians observing the volunteers, observed any sign or parameter that may indicate that the Product is unsafe for consumption at the tested low and high dose. The statistical analysis failed to detect any changes than could represent a safety risk.

Although the measured astaxanthin content in each tablet was lower than the targeted level, the actual high dose tested (19.25 mg), which did not raise any safety concern during the study, is 3.85 fold higher than the targeted 5 mg dose. As a result, *Haematococcus pluvialis* algae meal produced with Aquasearch's proprietary technology can be deemed safe for human consumption, when incorporated in dietary supplements containing equivalent levels of algae meal and astaxanthin to those tested in this study.



Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401



**Confidential**

Table 1. CLH "normal range" reference values.

| Variable | Reference Values: | |
|---|---|---|
| | Male | Female |
| Albumin | 3.5-5.0 g/dl | 3.5-5.0 g/dl |
| ALT (SGPT) | 0-40 IU/dl | 0-31 IU/dl |
| Bilirubin, Total | 0-1.2 mg.dl | 0-1.2 mg.dl |
| BUN | 6-19 mg/dl | 6-19 mg/dl |
| Creatinine | 0.5-1.2 mg/dl | 0.4-1.1 mg/dl |
| Cholesterol | <200 mg/dl | <200 mg/dl |
| Triglyceride | 0-200 mg/dl | 0-200 mg/dl |
| HDL Cholesterol | Average risk: 35-55 mg/dl | Average risk: 45-65 mg/dl |
| LDL | <130 mg/dl | <130 mg/dl |
| Chol/HDL ratio | Average risk: <4.5 | Average risk: <4.5 |
| LDL/HDL | Average risk: <2.0 | Average risk: <2.0 |
| Globulin | 2.5-4.0 g/dl | 2.5-4.0 g/dl |
| Glucose | 70-110 mg/dl | 70-110 mg/dl |
| LDH | 94-250 IU/l | 94-250 IU/l |
| Total Protein | 5.9-8.4 g/dl | 5.9-8.4 g/dl |
| WBC | 3.5-10.0 x $10^9$/l | 3.5-10.0 x $10^9$/l |
| RBC | 4.4-6.0 x $10^{12}$/l | 3.9-5.2 x $10^{12}$/l |
| Hemoglobin | 14-17 g/dl | 12-16 g/dl |
| Hematocrit | 41-51% | 35-46% |
| MCV | 80-100 fL | 80-100 fL |
| MCH | 27-33 pg | 27-33 pg |
| MCHC | 32-36 g/dl | 32-36 g/dl |
| RDW | 11-15% | 11-15% |
| Platelet Count | 150-450 x $10^9$/l | 150-450 x $10^9$/l |
| Neutrophils | 40-70% | 40-70% |
| Lymphocytes | 20-45% | 20-45% |
| Monocytes | 4-10% | 4-10% |
| Eosinophils | 0-6% | 0-6% |
| Basophils | 0-2% | 0-2% |
| RBC Morphology | n.a. | n.a. |
| PTT | 27-35 sec | 27-35 sec |
| Urine Color | n.a. | n.a. |
| Urine Appearance | n.a. | n.a. |
| Specific Gravity | 1.005-1.030 | 1.005-1.030 |
| Leukocyte esterase | Negative | Negative |
| Nitrite | Negative | Negative |
| PH | 5.0-7.5 | 5.0-7.5 |
| Protein | Negative | Negative |
| Glucose | Negative | Negative |
| Ketones | Negative | Negative |
| Urobilinogen | 0.2-1.0 EU/dl | 0.2-1.0 EU/dl |
| Bilirubin | Negative | Negative |
| Blood | Negative | Negative |

n.a. = not available.



Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

Confidential



000050

Table 2: Volunteer profile (age, sex) for each test group

| Low dose astaxanthin | | | High dose astaxanthin | | |
|---|---|---|---|---|---|
| Sex | Age | Volunteer # | Sex | Age | Volunteer # |
| M | 31 | 19 | M | 27 | 11 |
| M | 35 | 5 | M | 31 | 28 |
| M | 43 | 16 | M | 33 | 20 |
| M | 46 | 3 | M | 36 | 9 |
| M | 54 | 17 | M | 38 | 23 |
| M | 55 | 33 | M | 38 | 24 |
| M | 62 | 31 | M | 44 | 14 |
| F | 32 | 21 | M | 49 | 25 |
| F | 32 | 27 | F | 35 | 26 |
| F | 36 | 7 | F | 37 | 15 |
| F | 36 | 8 | F | 39 | 18 |
| F | 36 | 10 | F | 41 | 32 |
| F | 38 | 4 | F | 41 | 22 |
| F | 49 | 1 | F | 41 | 13 |
| F | 49 | 12 | F | 43 | 29 |
| F | 61 | 30 | F | 47 | 6 |
| | | | F | 55 | 2 |
| Average age (*std*) = 43 (*10*)<br>Head count = 16<br>(males = 7, females = 9) | | | Average age (*std*) = 40 (*7*)<br>Head count 17<br>(males = 8, females = 9) | | |
| All: average age (*std*) = 42 (*9*) (Min = 27, max = 62)<br>Head count all = 33 (males = 15, females = 18) | | | | | |



Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

**Confidential**



000051

Table 3. Summary statistics, Low Dose: Mean (standard deviation) of variables at T1-T4.

| Variables (from blood & urine samples) | T1 | | | T2 | | | T3 | | | T4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Avg | *Std* | N | Avg | *Std* | N | Avg | *Std* | N | Avg | *Std* | N |
| Albumin, 3.5-5.0 g/dL | **4.2** | *0.2* | 16 | **4.1** | *0.3* | 16 | **4.1** | *0.3* | 16 | **4.1** | *0.3* | 16 |
| ALT (SGPT), ♂ ≤40 IU/dL, ♀ ≤31 IU/dL | **22** | *10* | 16 | **19** | *11* | 16 | **21** | *11* | 16 | **21** | *10* | 16 |
| ALT (SGPT), only ♀ | **16** | *7* | 9 | **12** | *3* | 9 | **14** | *4* | 9 | **14** | *4* | 9 |
| ALT (SGPT), only ♂ | **30** | *7* | 7 | **28** | *11* | 7 | **30** | *9* | 7 | **30** | *8* | 7 |
| Bilirubin, ≤1.2 mg/dL | **0.6** | *0.3* | 16 | **0.6** | *0.3* | 15 | **0.6** | *0.2* | 16 | **0.6** | *0.2* | 16 |
| BUN, 6-19 mg/Dl | **17** | *5* | 16 | **17** | *5* | 16 | **15** | *5* | 16 | **14** | *5* | 16 |
| Creatinine, (♂ 0.5-1.2 mg/dL, ♀ 0.4-1.1 mg/dL) | **0.83** | *0.11* | 16 | **0.80** | *0.14* | 16 | **0.80** | *0.10* | 16 | **0.79** | *0.11* | 16 |
| Creatinine, only ♀ | **0.76** | *0.07* | 9 | **0.74** | *0.12* | 9 | **0.74** | *0.09* | 9 | **0.73** | *0.09* | 9 |
| Creatinine, only ♂ | **0.91** | *0.07* | 7 | **0.87** | *0.13* | 7 | **0.87** | *0.08* | 7 | **0.86** | *0.10* | 7 |
| Cholesterol, <200 mg/dL | **199** | *29* | 16 | **198** | *32* | 16 | **191** | *25* | 16 | **191** | *27* | 16 |
| Triglyceride, ≤200 mg/dL | **113** | *86* | 16 | **98** | *66* | 16 | **88** | *61* | 15 | **106** | *77* | 15 |
| HDL, ♂ 35-55 mg/dL, ♀ 45-65 mg/dL | **60** | *15* | 16 | **60** | *17* | 16 | **60** | *14* | 16 | **59** | *14* | 16 |
| HDL, only ♀ | **64** | *13* | 9 | **66** | *17* | 9 | **65** | *13* | 9 | **64** | *13* | 9 |
| HDL, only ♂ | **54** | *16* | 7 | **53** | *15* | 7 | **55** | *15* | 7 | **54** | *13* | 7 |
| LDL, <130 mg/dL | **117** | *26* | 16 | **118** | *28* | 16 | **114** | *25* | 15 | **112** | *26* | 15 |
| Cholesterol:HDL, <4.5 | **3.6** | *1.4* | 16 | **3.5** | *1.2* | 16 | **3.4** | *1.1* | 16 | **3.4** | *1.2* | 16 |
| LDL:HDL, <2.0 | **2.2** | *1.0* | 13 | **2.2** | *1.0* | 16 | **2.0** | *0.9* | 16 | **2.1** | *0.8* | 15 |
| Globulin, 2.5-4.0 g/dL | **3.3** | *0.4* | 16 | **3.4** | *0.4* | 16 | **3.3** | *0.4* | 16 | **3.0** | *0.4* | 16 |
| Glucose, 70-110 mg/dL | **94** | *17* | 16 | **91** | *9* | 16 | **95** | *6* | 16 | **92** | *10* | 16 |
| LDH, 94-250 IU/L | **173** | *39* | 16 | **167** | *28* | 16 | **163** | *25* | 16 | **160** | *24* | 16 |
| Total Protein, 5.9-8.4 g/dL | **7.5** | *0.5* | 16 | **7.5** | *0.4* | 16 | **7.4** | *0.6* | 16 | **7.3** | *0.5* | 16 |
| WBC, 3.4-10.0 x $10^9$/L | **6.6** | *2.0* | 16 | **6.1** | *1.9* | 16 | **6.0** | *2.0* | 16 | **6.2** | *1.8* | 16 |
| RBC, ♂ 4.4-6.0 x $10^{12}$/L, ♀ 3.9-5.2 x $10^{12}$/L | **4.6** | *0.5* | 16 | **4.5** | *0.4* | 16 | **4.6** | *0.5* | 16 | **4.6** | *0.5* | 16 |
| RBC, only ♀ | **4.30** | *0.37* | 9 | **4.32** | *0.21* | 9 | **4.31** | *0.33* | 9 | **4.25** | *0.30* | 9 |
| RBC, only ♂ | **4.92** | *0.40* | 7 | **4.83** | *0.42* | 7 | **4.89** | *0.46* | 7 | **5.02** | *0.38* | 7 |
| Hemoglobin, ♂ 14-17 g/dL, ♀ 12-16 g/dL | **14.2** | *1.2* | 16 | **14.3** | *1.1* | 16 | **14.1** | *1.2* | 16 | **14.1** | *1.4* | 16 |
| Hemoglobin, only ♀ | **13.5** | *0.7* | 9 | **13.6** | *0.5* | 9 | **13.4** | *0.9* | 9 | **13.2** | *0.9* | 9 |
| Hemoglobin, only ♂ | **15.2** | *1.1* | 7 | **15.1** | *1.1* | 7 | **15.0** | *1.1* | 7 | **15.2** | *1.0* | 7 |
| Hematocrit, ♂ 41-51%, ♀ 35-46% | **42** | *4* | 16 | **41** | *3* | 16 | **41** | *3* | 16 | **42** | *4* | 16 |
| Hematocrit, only ♀ | **39.2** | *2.2* | 9 | **39.5** | *1.4* | 9 | **39.2** | *2.0* | 9 | **38.9** | *2.2* | 9 |
| Hematocrit, only ♂ | **44.6** | *2.5* | 7 | **43.5** | *2.9* | 7 | **44.2** | *3.0* | 7 | **45.5** | *2.7* | 7 |
| Mean Corpuscular Volume, 80-100 fL | **88.7** | *2.4* | 16 | **88.7** | *2.4* | 16 | **88.8** | *2.3* | 16 | **89.2** | *2.2* | 16 |
| Mean Corpuscular Hemoglobin, 27-33 pg | **31.2** | *1.5* | 16 | **31.4** | *1.3* | 16 | **31.0** | *1.2* | 16 | **30.7** | *1.2* | 16 |
| MCH Concentration, 32-36 g/dL | **34.2** | *0.7* | 16 | **34.6** | *0.4* | 16 | **34.1** | *0.6* | 16 | **33.6** | *0.8* | 16 |
| Red cell Distribution Width, 11-15% | **12.8** | *0.8* | 16 | **12.9** | *0.7* | 16 | **12.8** | *0.7* | 16 | **12.7** | *0.5* | 16 |
| Platelet Count, 150-450 x $10^9$/L | **263** | *70* | 16 | **262** | *71* | 16 | **263** | *67* | 16 | **279** | *81* | 16 |
| Neutrophils, 40-70% | **61** | *8* | 16 | **56** | *8* | 16 | **57** | *10* | 16 | **58** | *8* | 16 |
| Lymphocytes, 20-45% | **29** | *7* | 16 | **33** | *7* | 16 | **32** | *9* | 16 | **31** | *7* | 16 |
| Monocytes, 4-10% | **6.4** | *1.4* | 16 | **7.3** | *1.7* | 16 | **6.8** | *1.6* | 16 | **7.0** | *1.7* | 16 |
| Eosinophils, 0-6% | **3.1** | *2.4* | 16 | **3.3** | *2.2* | 16 | **3.6** | *22.7* | 16 | **2.9** | *2.1* | 16 |
| Basophils, 0-2% | **1.0** | *0.4* | 15 | **1.0** | *0.4* | 16 | **0.8** | *0.5* | 16 | **0.8** | *0.5* | 16 |
| PTT, 27-35 sec | **29** | *3* | 16 | **30** | *2* | 16 | **30** | *2* | 16 | **29** | *2* | 16 |
| Urine specific gravity, 1.005-1.03 g/L | **1.020** | *0.006* | 16 | **1.021** | *0.006* | 16 | **1.020** | *0.007* | 16 | **1.016** | *0.006* | 16 |
| Urine pH, 5.0-7.5 | **5.8** | *0.9* | 16 | **5.7** | *0.9* | 16 | **5.8** | *0.9* | 16 | **5.4** | *0.7* | 16 |
| Urobilinogen, 0.2-1.0 EU/dL | **0.2** | *0* | 16 | **0.2** | *0* | 16 | **0.2** | *0* | 16 | **0.2** | *0* | 16 |



Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401
Confidential



000052

Table 4: Summary statistics, High Dose: Mean, standard deviation, and sample size at T1-T4.

| Variables (from blood & urine samples) | T1 | | | T2 | | | T3 | | | T4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Avg | *Std* | N | Avg | *Std* | N | Avg | *Std* | N | Avg | *Std* | N |
| Albumin, 3.5-5.0 g/dL | **4.2** | *0.3* | 17 | **4.1** | *0.3* | 17 | **4.1** | *0.3* | 17 | **4.1** | *0.3* | 17 |
| ALT (SGPT), ♂ ≤40 IU/dL, ♀ ≤31 IU/dL | **18** | *7* | 17 | **16** | *6* | 17 | **16** | *6* | 17 | **20** | *11* | 17 |
| ALT (SGPT), only ♀ | **17** | *5* | 9 | **14** | *4* | 9 | **15** | *5* | 9 | **17** | *7* | 9 |
| ALT (SGPT), only ♂ | **20** | *9* | 8 | **18** | *8* | 8 | **17** | *8* | 8 | **23** | *14* | 8 |
| Bilirubin, ≤1.2 mg/dL | **0.7** | *0.3* | 17 | **0.7** | *0.4* | 17 | **0.6** | *0.3* | 17 | **0.6** | *0.4* | 17 |
| BUN, 6-19 mg/Dl | **12** | *3* | 17 | **14** | *2* | 17 | **13** | *3* | 17 | **13** | *3* | 17 |
| Creatinine, (♂ 0.5-1.2 mg/dL, ♀ 0.4-1.1 mg/dL) | **0.88** | *0.13* | 17 | **0.82** | *0.14* | 17 | **0.84** | *0.16* | 17 | **0.82** | *0.13* | 17 |
| Creatinine, only ♀ | **0.81** | *0.15* | 9 | **0.73** | *0.12* | 9 | **0.74** | *0.17* | 9 | **0.74** | *0.13* | 9 |
| Creatinine, only ♂ | **0.95** | *0.05* | 8 | **0.91** | *0.08* | 8 | **0.94** | *0.07* | 8 | **0.91** | *0.06* | 8 |
| Cholesterol, <200 mg/dL | **192** | *39* | 17 | **191** | *36* | 17 | **183** | *32* | 17 | **187** | *38* | 17 |
| Triglycerides, ≤200 mg/dL | **100** | *46* | 17 | **98** | *56* | 17 | **88** | *47* | 17 | **87** | *40* | 17 |
| HDL, ♂ 35-55 mg/dL, ♀ 45-65 mg/dL | **56** | *9* | 17 | **54** | *10* | 17 | **56** | *11* | 17 | **57** | *11* | 17 |
| HDL, only ♀ | **59** | *8* | 9 | **58** | *8* | 9 | **58** | *6* | 9 | **60** | *11* | 9 |
| HDL, only ♂ | **53** | *10* | 8 | **50** | *11* | 8 | **54** | *15* | 8 | **54** | *11* | 8 |
| LDL, <130 mg/dL | **116** | *33* | 17 | **117** | *33* | 17 | **109** | *30* | 17 | **112** | *35* | 17 |
| Cholesterol:HDL, <4.5 | **3.5** | *0.9* | 17 | **3.6** | *1.0* | 17 | **3.4** | *1.0* | 17 | **3.4** | *1.1* | 17 |
| LDL:HDL, <2.0 | **2.1** | *0.7* | 17 | **2.2** | *0.8* | 17 | **2.1** | *0.8* | 17 | **2.1** | *0.9* | 17 |
| Globulin, 2.5-4.0 g/dL | **3.2** | *0.3* | 17 | **3.2** | *0.4* | 17 | **3.3** | *0.3* | 17 | **3.2** | *0.4* | 17 |
| Glucose, 70-110 mg/dL | **97** | *10* | 17 | **96** | *10* | 17 | **97** | *8* | 17 | **94** | *8* | 17 |
| LDH, 94-250 IU/L | **160** | *31* | 17 | **157** | *29* | 17 | **157** | *25* | 17 | **153** | *27* | 17 |
| Total Protein, 5.9-8.4 g/dL | **7.5** | *0.5* | 17 | **7.3** | *0.5* | 17 | **7.4** | *0.5* | 17 | **7.3** | *0.6* | 17 |
| WBC, 3.4-10.0 x $10^9$/L | **5.6** | *1.1* | 17 | **5.5** | *1.2* | 17 | **5.7** | *1.2* | 17 | **5.6** | *1.4* | 17 |
| RBC, ♂ 4.4-6.0 x $10^{12}$/L, ♀ 3.9-5.2 x $10^{12}$/L | **4.6** | *0.4* | 17 | **4.5** | *0.4* | 17 | **4.6** | *0.4* | 17 | **4.6** | *0.3* | 17 |
| RBC, only ♀ | **4.31** | *0.34* | 9 | **4.28** | *0.31* | 9 | **4.29** | *0.28* | 9 | **4.44** | *0.24* | 9 |
| RBC, only ♂ | **4.93** | *0.25* | 8 | **4.85** | *0.26* | 8 | **4.91** | *0.29* | 8 | **4.91** | *0.23* | 7 |
| Hemoglobin, ♂ 14-17 g/dL, ♀ 12-16 g/dL | **14.7** | *1.4* | 17 | **14.7** | *1.3* | 17 | **14.5** | *1.3* | 17 | **14.7** | *1.1* | 17 |
| Hemoglobin, only ♀ | **13.7** | *0.9* | 9 | **13.8** | *0.8* | 9 | **13.6** | *0.6* | 9 | **14.1** | *0.7* | 9 |
| Hemoglobin, only ♂ | **15.9** | *1.0* | 8 | **15.7** | *1.2* | 8 | **15.6** | *1.0* | 8 | **15.4** | *1.0* | 8 |
| Hematocrit, ♂ 41-51%, ♀ 35-46% | **43** | *4* | 17 | **42** | *4* | 17 | **43** | *4* | 17 | **43** | *3* | 17 |
| Hematocrit, only ♀ | **40.1** | *2.5* | 9 | **39.8** | *2.4* | 9 | **39.8** | *1.8* | 9 | **41.6** | *2* | 9 |
| Hematocrit, only ♂ | **45.9** | *2.7* | 8 | **45.1** | *2.8* | 8 | **45.7** | *2.8* | 8 | **45.5** | *2.9* | 8 |
| Mean Corpuscular Volume, 80-100 fL | **95.3** | *2.6* | 17 | **95.1** | *2.8* | 17 | **95.0** | *2.5* | 17 | **95.4** | *2.6* | 17 |
| Mean Corpuscular Hemoglobin, 27-33 pg | **32.0** | *1.6* | 17 | **32.3** | *1.5* | 17 | **31.8** | *1.6* | 17 | **31.6** | *1.6* | 17 |
| MCH Concentration, 32-36 g/dL | **34.4** | *0.5* | 17 | **34.7** | *0.3* | 17 | **34.1** | *0.7* | 17 | **33.8** | *0.8* | 17 |
| Red cell Distribution Width, 11-15% | **12.9** | *0.6* | 17 | **12.8** | *0.5* | 17 | **12.7** | *0.5* | 17 | **12.6** | *0.5* | 17 |
| Platelet Count, 150-450 x $10^9$/L | **238** | *52* | 17 | **239** | *54* | 17 | **243** | *51* | 17 | **247** | *56* | 17 |
| Neutrophils, 40-70% | **56** | *6* | 17 | **55** | *8* | 17 | **57** | *8* | 17 | **54** | *9* | 17 |
| Lymphocytes, 20-45% | **33** | *4* | 17 | **33** | *7* | 17 | **31** | *6* | 17 | **34** | *8* | 17 |
| Monocytes, 4-10% | **7.8** | *2.1* | 17 | **7.9** | *2.2* | 17 | **8.2** | *2.3* | 17 | **7.9** | *2.1* | 17 |
| Eosinophils, 0-6% | **2.9** | *1.9* | 17 | **3.2** | *2.0* | 17 | **2.9** | *2.0* | 17 | **3.1** | *1.9* | 17 |
| Basophils, 0-2% | **0.9** | *0.3* | 17 | **0.9** | *0.4* | 17 | **0.8** | *0.5* | 17 | **0.8** | *0.5* | 17 |
| PTT, 27-35 sec | **29** | *2* | 17 | **30** | *2* | 17 | **30** | *2* | 17 | **31** | *2* | 17 |
| Urine specific gravity, 1.005-1.03 g/L | **1.016** | *0.006* | 17 | **1.019** | *0.007* | 17 | **1.016** | *0.008* | 17 | **1.015** | *0.007* | 17 |
| Urine pH, 5.0-7.5 | **6.2** | *1.1* | 16 | **5.7** | *0.9* | 17 | **6.2** | 1.0 | 17 | **5.9** | *1.0* | 17 |
| Urobilinogen, 0.2-1.0 EU/dL | **0.2** | *0* | 17 | **0.2** | *0* | 17 | **0.2** | 0 | 17 | **0.2** | *0* | 17 |



Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401



**Confidential**

## RD.0100.001. Appendix A: Composition and analysis of tablets

Table 1: Composition of the astaxanthin hard tablets

| Ingredient | Total weight (mg) | % inclusion |
|---|---|---|
| Algal meal powder (Lot 990610Mix) | 228.27 | 29.84% |
| Ascorbyl palmitate | 0.459 | 0.06% |
| Vitamin E (Covitol-700) | 0.459 | 0.06% |
| Dicalcium Phosphate (unmilled) | 347.157 | 45.38% |
| Hydrogenated vegetable oil | 78.18 | 10.22% |
| Magnesium stearate | 10.5 | 1.37% |
| Microcrystalline cellulose | 100 | 13.07% |
| Total | 765 | 100% |

CONFIDENTIAL

Table 2: Calculated nutrient composition of tablets in safety trial.

| Nutrients | Amount per tablet | Unit | % of daily value | Daily value | Unit |
|---|---|---|---|---|---|
| Total calories | 1.78 | Cal | 0.09% | 2000 | Cal |
| Calories from fat | 1.10 | Cal | | n.a.* | |
| Total fat | 122 | Mg | 0.19% | 65 | g |
| Saturated fat | 85 | Mg | 0.43% | 20 | g |
| Polyunsaturated fat | 11 | Mg | | n.a.* | |
| Cholesterol | 0 | Mg | 0.00% | 300 | mg |
| Sodium | 1.2 | Mg | 0.05% | 2400 | mg |
| Total carbohydrate | 127 | Mg | 0.04% | 300 | g |
| Dietary fiber | 160 | Mg | 0.64% | 25 | g |
| Sugars | 0 | Mg | | n.a.* | |
| Protein | 41 | Mg | 0.08% | 50 | g |
| Vitamin A | 50 | IU | 1.00% | 5000 | IU |
| Vitamin C | 0.3 | Mg | 0.46% | 60 | mg |
| Calcium | 77 | Mg | 7.66% | 1000 | mg |
| Iron | 0.2 | Mg | 1.12% | 18 | mg |
| Vitamin E | 0.4 | Mg | 4.13% | 10 | mg |
| Total carotenoids | 5.9 | Mg | | n.a.* | |
| Total astaxanthin | 5.0 | Mg | | n.a.* | |

*n.a. = not applicable

Table 3. Hard tablet analysis results

| Parameter | |
|---|---|
| Aerobic plate count | < 1,000 CFU/g |
| Total astaxanthin | 3.8 mg/tablet |
| Average weight | 765mg/tablet |



Aquasearch Inc.,©
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

RD.0100.001. Appendix B.

Aquasearch Inc.
bringing the oceans to life

# *A human safety trial of natural astaxanthin from Haematococcus pluvialis algae*

## INFORMED CONSENT AGREEMENT

Participant name:_____

*For further details, contact:*
Aquasearch Inc.,
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

Confidential

## INTRODUCTION

This is a clinical trial (a type of research study). Clinical trials include only patients who choose to take part. Please take your time to make your decision.

You are being asked to take part in this study because you are an adult (over 18 years of age), in good health, and not regularly taking any medications nor any dietary supplements that are high in carotenoids (i. e., less than 3 mg beta-carotene or other carotenoids daily) or in vitamin E (i. e., less that 400 IU vitamin E daily).

## WHY IS THIS STUDY BEING DONE?

- The purpose of this study is to evaluate the human tolerance of **astaxanthin from a natural source** (the freshwater alga *Haematococcus pluvialis*) and see what effects (good and bad) it may have on you and your health.
- This research is being done because astaxanthin is known from *in vitro* tests and from animal model studies to have powerful antioxidant properties, and has not been found to have any indication of risks to humans. We would like to evaluate the effects of astaxanthin from a natural source (*Haematococcus pluvialis*) in humans.

## HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

- About 30 people will take part in this study.

*For further details, contact:*
Aquasearch Inc.,
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

## WHAT IS INVOLVED IN THE STUDY?

- You will be "randomized" into one of the study groups described below. Randomization means that you are put into a group by chance. It is like flipping a coin. You will have a one-in-two chance of being placed in any particular group.

- There will be two groups of subjects. The first group will be asked to consume astaxanthin at **5 mg per day for four weeks**. The second group will be asked to consume astaxanthin at **25 mg per day for four weeks**.

- Prior to the start of the study, you will be asked to report to a clinical laboratory where a complete physical examination will be carried out, including evaluation of skin color, and blood and urine samples taken for standard clinical analyses (blood and urine tests). This **first physical examination** and **first set of clinical analyses** will determine if you are eligible for this study. If the results of the examination and clinical chemistry analyses fall within ranges accepted as normal, you will be considered eligible for the full study. These medical evaluations are all free, and the results will be provided if you request them.

- Approximately one week later, the blood and urine testing will be repeated. The results of this **second set of clinical analyses**, combined with the first set, will provide a clinical chemistry baseline for this study.

- You will then be asked to consume natural astaxanthin produced from *Haematococcus pluvialis* alga, which has been processed into a tablet form. The amounts of astaxanthin to be taken daily will be either 5 mg or 25 mg. You will consume the appropriate number of tablets daily, together with your evening meal, for a period of four weeks (28 days).

- Three to seven days after the start of the study (i. e., three to seven days after your first consumption of the astaxanthin tablets), you will undergo a **second physical examination** and **third set of clinical analyses** (blood and urine tests).

- At the end of the study (i. e., on the 29th day after you first began consuming the astaxanthin tablets), you will undergo a **third physical examination** and a **fourth set of clinical analyses** (blood and urine tests).

*For further details, contact:*
Aquasearch Inc.,
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

## WHAT TESTS AND PROCEDURES WILL I UNDERGO DURING THE STUDY?

If you take part in this study, you will have the following tests and procedures. These procedures are routine procedures that are usually done as part of a complete physical examination. None of these procedures are experimental. You can obtain more information about these routine tests by speaking with the physician who will conduct the physical examinations during this study or with your personal physician.

- **Clinical samples.** Blood and urine samples will be taken four times during the research study as described above. Before samples are taken, a 12-hour (minimum) fast is needed. You may have dinner as usual the night before samples are to be taken. Only water is allowed after dinner. No alcohol should be ingested 24 hours before specimen collection. Samples will be obtained in the morning. Blood will be drawn using routine venipuncture procedures. Each sample collection will include one 10-mL serum separator tube (SST) and one 4.5 mL sodium citrate tube (for blood analyses), and one 5-10 mL urine sample. Breakfast will be provided after samples are taken.

- The following blood tests will be performed. **Blood chemistry analyses** will include serum glutamate pyruvate transaminase (SGPT), glucose, lactate dehydrogenase (LDH), total protein, total bilirubin, blood urea nitrogen (BUN), creatinine, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol (calculated), albumin, and globulin. A **complete blood count** (CBC) will be performed, to include white blood count (WBC), red blood count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count, neutrophil (segs), lymphocytes, monocytes, eosinophils, basophils, and red blood cell morphology. Finally, a **coagulation test** (activated partial thromboplastin time, PTT) will be performed. At each blood sampling,

- The **urinalysis tests** will include color, appearance, specific gravity, leukocyte esterase, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, and blood.

*For further details, contact:*
Aquasearch Inc.,
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

## HOW LONG WILL I BE IN THE STUDY?

You will be in the study for four weeks, plus the time necessary for the initial screening of volunteers. Some of the results from the analyses may not be available until a few days after completion of the test. A physician will review these analyses and may want to examine you again, within 4 weeks after completion of the study.

## WHAT ARE THE RISKS OF THE STUDY?

While on the study, you may experience the following harmless and temporary conditions. You should discuss these with the researcher and/or your regular doctor.

*Possible but not serious:*

- Carotenodermia or reddening of the skin, particularly of the palms.
- Reddening of stools.

## ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

If you agree to take part in this study, there may or may not be direct medical benefit to you. We hope the information learned from this study will benefit other individuals in the future. You will have access to the results of the medical examinations and tests performed as part of this study.

## WHAT ABOUT CONFIDENTIALITY?

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Food and Drug Administration.

*For further details, contact:*
Aquasearch Inc.,
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

## WHAT ARE THE COSTS?

You will not be charged for any of the medical examinations and tests undergone. Even if the results of your initial examination and clinical tests show that you are not eligible to participate further in the study, you will not be charged. You will receive no payment for taking part in this study. However, a small compensation is offered as an incentive to those who complete the study.

## WHAT ARE MY RIGHTS AS A PARTICIPANT?

Taking part in this study is voluntary. You may choose to not take part, or may leave the study at any time. Leaving the study will not result in any penalty. However an incentive is offered to those who complete the study. We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study.

## WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related problem, you may contact one of the following researchers: Dr. John Dore, Dr. Miguel Olaizola, or Dr. Mia Unson at (808)326-9301. For other questions about health-related issues, you may wish to contact your personal physician.

## WHERE CAN I GET MORE INFORMATION?

For specific information about the study, contact the researcher(s) one of the following researchers: Dr. John Dore, Dr. Miguel Olaizola, or Dr. Mia Unson at (808)326-9301. You may also obtain **independent information** about the antioxidant role of carotenoids from these websites and others:

- The VERIS Research Information Service (**http://www.veris-online.org**) is a not-for-profit corporation that strives to provide a responsible source of information on the role of nutrition in health--with an emphasis on antioxidants. The information included in VERIS publications is derived from technical articles that have been published in peer-reviewed journals, and every effort is made to present the material accurately and objectively. VERIS disseminates current research data on the benefits of vitamin E, carotenoids and other antioxidants.

*For further details, contact:*
Aquasearch Inc.,
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

- Carotenoid information from the University of Berne, Switzerland (**http://dcb-carot.unibe.ch/carotint.htm**)
- General health information can also be found at several websites, including HealthFinder (a service of the U. S. Department of Health and Human Services, at **http://www.healthfinder.gov**), MedicineNet (**http://www.medicinenet.com**), and InteliHealth from the Johns Hopkins University (**http://www.intelihealth.com/IH/ihtIH**).

**You will get a copy of this form.**

**SIGNATURE**

**I agree to take part in this study.**

**Participant's signature**_____

**Date** _____

**Participant's full legal name (please print clearly)** _____

*For further details, contact:*
Aquasearch Inc.,
73-4460 Queen Kaahumanu Highway,
Suite 110, Kailua-Kona, HI 96740, USA
Tel: 808-326 9301, Fax: 808-326 9401

SAMPLE

RD.0160.001 Appendix C

LABORATORY REPORT

Clinical Laboratories of Hawaii, LLC
(808) 677-7999 (SC)

OKAZAKI, TRUDY (14921)
91-2135 FT WEAVER RD STE 300
EWA BEACH, HI 96706
808-677-7999

Ordering Phys: AQUASEARCH

1

| PATIENT NUMBER [redacted] | PAT [redacted] | AGE 49 | SEX F | REPORT DATE 10/15/1999 | TIME 6:32 AM |
|---|---|---|---|---|---|

REMARKS
Patient Tel No: SSN: BD: [redacted] YOP



Collected: 10/14/1999 6:25 Accession: [redacted]

| | RESULTS | UNITS | REFERENCE RANGE | LC |
|---|---|---|---|---|
| Albumin | 4.1 | g/dL | (3.5-5.0) | A |
| ALT (SGPT) | 13 | IU/L | (0-31) | A |
| Bilirubin, Total | 1.0 | mg/dL | (0-1.2) | A |
| BUN | 11 | mg/dL | (6-19) | A |
| Creatinine | 0.9 | mg/dL | (0.4-1.1) | A |
| Lipid Profile | | | | A |
| Cholesterol | 168 | mg/dL | (<200) | |
| Triglyceride | 57 | mg/dL | (0-200) | |
| HDL Cholesterol | 72 | mg/dL | | |
| | HDL Reference Range (female): | | | |
| | Favorable: >65 | | | |
| | Average risk: 45-65 | | | |
| | Above average risk: <45 | | | |
| LDL | 85 | mg/dL | (<130) | |
| CHOL/HDL Ratio | 2.33 L | | (2.90-4.40) | |
| | Chol/HDL ratio interpretation: | | | |
| | Ideal: <3.5 | | | |
| | Average risk: <4.5 | | | |
| | 2x average risk: 10.0 | | | |
| | 3x average risk: 20.0 | | | |
| LDL/HDL | 1.2 | | | |
| | LDL/HDL ratio interpretation: | | | |
| | Ideal: <1.0 | | | |
| | Average risk: <2.0 | | | |
| | 2x Average risk: 6.0 | | | |
| | 3x Average risk: 8.0 | | | |
| Globulin | 2.6 | g/dL | (2.5-4.0) | A |
| Glucose | 86 | mg/dL | (70-110) | A |

CONTINUED....

000062

SAMPLE

RD.0100.001 Appendix C

KAZAKI, TRUDY (14921)
[redacted]-2135 FT WEAVER RD STE 300
EWA BEACH, HI 96706
808-677-7999

Ordering Phys: AQUASEARCH

LABORATORY REPORT

Clinical Laboratories of Hawaii, LLC
(808) 677-7999 (SC)

PAT [redacted] | PA [redacted] | A [redacted] | SEX | REPORT DATE [redacted] | TIME 6:32 AM

REMARKS Patient Tel No: SSN: BD: [redacted] YOP



Collected: 10/14/1999 6:25 Accession: [redacted]

| | RESULTS | UNITS | REFERENCE RANGE | LOC |
|---|---|---|---|---|
| Glucose (continued) | | | | |
| **LD (LDH)** | 149 | IU/L | (94-250) | A |
| **Total Protein** | 6.7 | g/dL | (5.9-8.4) | A |
| **Automated Bld Cnt** | | | | A |
| WBC | 7.2 | 10(9)/L | (3.5-10.0) | |
| RBC | 4.50 | 10(12)/L | (3.9-5.2) | |
| Hemoglobin | 14.2 | g/dL | (12-16) | |
| Hematocrit | 42.2 | % | (35-46) | |
| MCV | 93.6 | fL | (80-100) | |
| MCH | 31.5 | pg | (27-33) | |
| MCHC | 33.6 | g/dL | (32-36) | |
| RDW | 12.7 | % | (11-15) | |
| Platelet Count | 241 | 10(9)/L | (150-450) | |
| **Differential** | | | | A |
| Diff Method | Auto | | | |
| Neutrophils | 49 | % | (40-70) | |
| Lymphs | 38 | % | (20-45) | |
| Monocytes | 7 | % | (4-10) | |
| Eosinophils | 5 | % | (0-6) | |
| Basophils | 1 | % | (0-2) | |
| RBC Morphology | Normal | | | |
| **PTT** | 30 | sec | (27-35) | A |
| **Urine Macroscopic** | | | | A |
| Color | Yellow | | | |
| Appearance | Clear | | | |
| Specific Gravity | 1.008 | | (1.005-1.030) | |
| Leukocyte Esterase | Negative | | (NEG) | |
| Nitrite | Negative | | (NEG) | |
| PH | 5.0 | | (5.0-7.5) | |
| Protein | Negative | mg/dL | (NEG) | |
| Glucose | Negative | mg/dL | (NEG) | |
| Ketones | Negative | mg/dL | (NEG) | |
| Urobilinogen | 0.2 | EU/dL | (0.2-1.0) | |
| Bilirubin | Negative | | (NEG) | |
| Blood | Negative | | (NEG) | |

CONTINUED....

000063

SAMPLE

RD.0100.001

Appendix C

LABORATORY REPORT

KAZAKI, TRUDY (14921)
91-2135 FT WEAVER RD STE 300
EWA BEACH, HI 96706
808-677-7999

Ordering Phys: AQUASEARCH

Clinical Laboratories of Hawaii, LLC
(808) 677-7999 (SC)

| TIEN [redacted] | PAT [redacted] | AGE 49 | SEX F | REPORT DATE 10/15/1999 | TIME 6:32 AM |
|---|---|---|---|---|---|

MARKS
Patient Tel No: SSN: BD: [redacted] YOP



Collected: 10/14/1999 6:25 Accession: H81657/C935938

| | RESULTS | UNITS | REFERENCE RANGE | LOC |
|---|---|---|---|---|

**ABNORMAL SUMMARY**

| | | | | |
|---|---|---|---|---|
| **Lipid Profile** | | | | |
| CHOL/HDL Ratio | 2.33 | L | (2.90-4.40) | |
| Chol/HDL ratio interpretation: | | | | |
| Ideal: | <3.5 | | | |
| Average risk: | <4.5 | | | |
| 2x average risk: | 10.0 | | | |
| 3x average risk: | 20.0 | | | |

PLEASE NOTE: The abnormal summary is supplied as a tool for identifying abnormal results. All results must still be reviewed as some abnormal results will not be included due to their interpretative or textual nature.

**FINAL REPORT**

[redacted]

Testing Locations:

Additional Copy. Test(s) originally ordered by: AQUASEARCH C/O DR MARK KUGE

000004

RD.0100.001 Appendix C

# SAMPLE

KONA-KOHALA MEDICAL ASSOC., INC.
75-137 HUALALAI ROAD
KAILUA-KONA, HI 96740

COMPANY Aquasearch

Pre-emp ☐ BMC ☐ X-Ray ☐
Annual ☐ Crane ☐ Chest ☐ Back ☐
Exec. ☐ Other

Position Applying for:

NAME: [redacted] (LAST FIRST MIDDLE)
Res. Address.: [redacted]
Res. Tel. No.: [redacted]
Birthdate [redacted] Age [redacted]
☒ Male ☐ Female S.S. # [redacted]

## PREVIOUS HEALTH HISTORY

| | HAVE YOU EVER HAD: | YES | NO | YEAR |
|---|---|---|---|---|
| 1 | Head Injuries | | X | |
| 2 | Headaches, Dizziness, Fainting | | X | |
| 3 | Emotional or Nervous Trouble | | X | |
| 4 | Convulsions or Loss of Consciousness | | X | |
| 5 | Asthma, Hay Fever, Sinus Trouble | | X | |
| 6 | Allergies (Including Medication) | | X | |
| 7 | Eye Trouble, Poor Vision | | X | |
| 8 | Ear Trouble, Poor Hearing | | X | |
| 9 | Frequent Sore Throat | | X | |
| 10 | Diabetes | | X | |
| 11 | Tuberculosis | | X | |
| 12 | Pneumonia or Pleurisy | | X | |
| 13 | Cough or Chest Pain | | X | |
| 14 | Heart Trouble or Shortness of Breath | | X | |
| 15 | Swelling of Legs or Ankles | | X | |
| 16 | High or Low Blood Pressure | | X | |
| 17 | Stroke, Paralysis, Weakness | | X | |
| 18 | Stomach Trouble, Ulcers, Indigestion | | X | |
| 19 | Liver or Gall Bladder Trouble | | X | |
| 20 | Constipation or Diarrhea | | X | |
| 21 | Rectal or Hemorrhoid Trouble | | X | |
| 22 | Kidney Trouble or Stones | | X | |
| 23 | Hernia | | X | |
| 24 | Varicose Veins | | X | |
| 25 | Rheumatism, Arthritis, Gout | | X | |
| 26 | Venereal Disease | | X | |
| 27 | Deformity Amputation | | X | |
| 28 | Back Trouble | | X | |
| 29 | Operations, Injuries, Fractures | | X | |
| 30 | Hospital Care | | X | |
| 31 | Scars, Identifying Marks | | X | |
| 32 | Skin Trouble | | X | |
| 33 | Recent Gain or Loss of Weight | | X | |
| 34 | Excess Alcohol Ingestion | | X | |
| 35 | Cancer | | X | |
| 36 | Persistent Fever | | X | |
| 37 | Chronic Fatigue | | X | |
| 38 | Hepatitis | | X | |
| 39 | Chemical Exposure | | X | |
| 40 | A Rejection on Life or Health Ins. | | X | |
| 41 | A Rejection by Military Service | | X | |
| 42 | Workmen's Compensation Claim | | X | |
| 43 | Do You Use Cocaine, LSD, Marijuana or Other Drugs? | | X | |
| 44 | Do you smoke? | | X | |
| 45 | Are you taking medication? | | X | |
| | **FAMILY HISTORY HAS ANYONE HAD OR HAVE** | | | |
| 46 | Tuberculosis | X | | |
| 47 | Diabetes | | X | |
| 48 | Heart Disease | | X | |
| 49 | Epilepsy | | X | |
| | **FEMALE APPLICANTS:** | | | |
| 51 | Womb or Ovary Trouble? | | | |
| 52 | Last Menstrual Period? | | | |

REMARKS—INDICATE NO.
4/6 months had TB (pt ⊖ APD's)

| | EXAMINATION | | |
|---|---|---|---|
| 48 | Height 43 Weight | in. | 155.5 lbs |
| 50 | Blood Pressure | 120 | 72 |
| 51 | Pulse Rate/after exercise | 60 | |
| 52 | Distant Vision Corr./Uncorr. | R 20/25 | L 20/20 |
| 53 | Near Vision Corr./Uncorr. | R 20/20 | L 20/20 |
| | OT 92 | NORMAL | ABNORMAL |
| 54 | Ishihara Color Test | ✓ | |
| 55 | Depth Perception | ✓ | |
| 56 | General Appearance | ✓ | |
| 57 | Skin | ✓ | |
| 58 | Eyes | ✓ | |
| 59 | Visual Fields | ✓ | |
| 60 | Ears and Nose | ✓ | |
| 61 | Mouth, Throat and Teeth | ✓ | |
| 62 | Neck and Head | ✓ | |
| 63 | Chest and Lungs | | X |
| 64 | Breasts | ✓ | |
| 65 | Heart | ✓ | |
| 66 | Abdomen | ✓ | |
| 67 | Back and Flexibility | ✓ | |
| 68 | Hernia | ✓ | |
| 69 | Extremities | ✓ | |
| 70 | Reflexes | | |
| 71 | Rectum | | |
| 72 | Genitalia | ✓ | |
| 73 | Pelvic Exam | | |
| 74 | Urine: Sugar/Albumin | | |
| 75 | Urine: Spec. Gr. | | |
| | Urine: Micro | | |
| | Hemoglobin | | |
| 78 | White Blood Count | | |
| 79 | Differential | | |
| 80 | Chest X-ray/PPD | | |
| 81 | Electrocardiogram | | |
| 82 | Other | | |

I have answered the above questions to the best of my knowledge. I hereby authorize Kona-Kohala Medical Associates, Inc. to forward the full results of my medical history to Aquasearch Inc.

Signature [signature] Date 9/9/99

**AUDIOGRAMS**

| | 500 | 1000 | 2000 | 3000 | 4000 | 6000 |
|---|---|---|---|---|---|---|
| R | | | | | | |
| L | | | | | | |

**PFT**

| | Act. | Pred. Norm | % |
|---|---|---|---|
| FVC | | | |
| $FEV_1$ | | | |

**TONOMETRY**

| mm | Hg |
|---|---|
| R | |
| L | |

**PHYSICIAN'S REMARKS AND REPORTS**

pectus excavatum - otherwise chest OK

**FOR BMC ONLY—MEDICAL EXAMINER'S CERTIFICATE** I certify that I have examined:

Driver's name (Print) Soc. Sec. No.

In accordance with the Motor Carrier Regulations (391.41-391.49) and with the knowledge of his duties, I find him qualified under the regulations.
☐ Qualified only when wearing corrective lenses.
☐ Qualified only when wearing a hearing aid.
A completed examination form for this person is on file in my office.

000065

New Certification ☐
Recertification ☐

(Signature of driver)

☒ No limitation
☐ Limitation - See Remarks

Signature of Examining Physician

RD.0100.0001

## Progress Notes

[redacted]

SEP 09 1999 OT 975 P 60 BP 120/72 (L) Wt 155 1/2 #
0922 Aquasearch PE
Meds-0
NKDA
pectus excavatum otherwise OK
See PMH form
PMD (Chas see Pekala)

SEP 23 1999 OT 974 P 56 BP 126/70 (L) Wt 154 1/2 #
0914 FU
- sl. color stools, odor urine

[redacted] 9-23-99

S: Feels fine. Has no symptoms. Has noted a slight coloration to the stool and slight odor to the urine but otherwise has noted no change.
O: Exam is normal. There is no skin coloration change. Blood tests are reviewed, all normal and showing no serial change.
P: Proceed witih Protocol. ES:mk

OCT 14 1999 OT 974 P 52 BP 126/74 (L) Wt 154 #
1020 FU
Distant vision 3 OD 20/25 OS 20/20
Near " 3 OD 20/20 OS 20/20
Color- " nl

[redacted] 10-14-99

S: A 33 year old male comes in for Astaxanthin trial protocol. He has felt fine. Has noticed no change other than for a slight increased orangeness to his stool and urine. Has noted no skin change or other problems.
O: Vitals stable, afebrile. SKIN: Without jaundice. EYES: Without icterus. ENT: Negative. NECK: Without bruits, thyromegaly. LUNGS: Clear. HEART: Normal. CHEST: With pectus excavatum. ABD: Without hepatosplenomegaly. EXT: Without edema, cyanosis.
A: Unremarkable exam.
P: Proceed with protocol. RP:mk

000066

## Summary of statistical analysis: Aquasearch safety study. Report # RD.0100.001

Summary probabilities: 2-way ANOVA, proportional replication, for two comparisons: T1T2vsT4 AND T1T2vsT3, at each dose.

| PARAMETER | | LOW DOSE: avg | std | F(a/int) | p | HIGH DOSE: avg | std | F(a/int) | p | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Albumin [g/dL],** 3.5-5.0 | T1T2 | 4.15 | 0.26 | | | 4.17 | 0.33 | | | |
| | T3 | 4.09 | 0.34 | 1.58 | | 4.12 | 0.32 | 1.49 | | |
| | T4 | 4.09 | 0.30 | 1.86 | | 4.12 | 0.32 | 1.97 | | |
| **ALT (serum glutamate pyruvate transaminase, SGPT) [IU/L],** 0-31 | T1T2 | 20.34 | 10.50 | | | 17.21 | 6.90 | | | |
| | T3 | 20.75 | 10.59 | 0.08 | | 16.06 | 6.34 | 0.69 | | |
| | T4 | 21.31 | 9.86 | 0.63 | | 20.12 | 11.03 | 2.26 | | |
| **Bilirubin, total [mg/dL],** 0-1.2 | T1T2 | 0.60 | 0.27 | | | 0.68 | 0.35 | | | |
| | T3 | 0.56 | 0.18 | 1.10 | | 0.62 | 0.34 | 1.58 | | |
| | T4 | 0.61 | 0.23 | 0.01 | | 0.64 | 0.37 | 0.55 | | |
| **BUN (blood urea nitrogen) [mg/dL],** 6-19 | T1T2 | 16.72 | 4.73 | | | 12.88 | 2.94 | | | A small significant decrease at T4 of low dose, |
| | T3 | 15.19 | 4.97 | 2.67 | | 12.82 | 3.00 | 0.01 | | but a small increase at the high dose |
| | T4 | 14.25 | 4.55 | 18.35 | **<0.001** | 13.06 | 2.68 | 0.11 | | therefore no significance or safety concern |
| **Creatinine [mg/dL],** 0.4-1.1 | T1T2 | 0.81 | 0.12 | | | 0.85 | 0.14 | | | |
| | T3 | 0.80 | 0.10 | 0.35 | | 0.84 | 0.16 | 0.28 | | |
| | T4 | 0.79 | 0.11 | 1.87 | | 0.82 | 0.13 | 1.99 | | |
| **Cholesterol [mg/dL],** <200 | T1T2 | 198 | 30 | | | 192 | 37 | | | A small significant decrease at T3 of the high dose |
| | T3 | 191 | 25 | 2.80 | | 183 | 32 | 12.49 | **<0.005** | followed by a small increase at T4. |
| | T4 | 191 | 27 | 6.44 | | 187 | 38 | 1.30 | | Therefore, no significance. No safety concern. |
| **Triglyceride [mg/dL],** 0-200 | T1T2 | 108 | 77 | | | 99.2 | 50.2 | | | A small significant decrease at T3 of the low dose |
| | T3 | 88 | 61 | 8.13 | **<0.025** | 88.1 | 47.0 | 1.13 | | but no difference at T4. |
| | T4 | 106 | 77 | 0.04 | | 86.8 | 39.6 | 1.33 | | Therefore, no significance. No safety concern. |
| **HDL cholesterol [mg/dL]** favorable >65 | T1T2 | 60.2 | 14.9 | | | 55.3 | 9.1 | | | |
| | T3 | 60.3 | 14.1 | | | 56.2 | 10.8 | 0.50 | | |
| | T4 | 59.3 | 13.8 | | | 57.3 | 11.1 | 1.59 | | |
| **Cholesterol/HDL ratio:** ideal <3.5 | T1T2 | 3.57 | 1.38 | | | 3.56 | 0.85 | | | A small significant decrease at T3 of the high dose, |
| | T3 | 3.39 | 1.14 | 3.21 | | 3.38 | 0.98 | 9.67 | **<0.01** | confirmed by a stabilisation at T4. A small NS decrease |
| | T4 | 3.45 | 1.22 | 1.38 | | 3.39 | 1.07 | 2.69 | | at T3 and T4 of the low dose: possibly a positive effect |
| | | | | | | | | | | No safety concern. |
| **LDL cholesterol [mg/dL],** <130 | T1T2 | 117 | 27 | | | 116 | 33 | | | A small significant decrease at T3 of the high dose, |
| | T3 | 114 | 25 | 1.05 | | 109 | 30 | 5.04 | **<0.05** | confirmed by a stabilisation at T4. A small NS decrease |
| | T4 | 112 | 26 | 2.63 | | 112 | 35 | 1.48 | | at T3 and T4 of the low dose: possibly a positive effect |
| | | | | | | | | | | No safety concern. |
| **LDL:HDL, ideal <1.0** | T1T2 | 2.16 | 0.96 | | | 2.16 | 0.76 | | | |
| | T3 | 2.03 | 0.88 | 0.48 | | 2.09 | 0.84 | 1.25 | | |
| | T4 | 2.06 | 0.83 | 1.41 | | 2.07 | 0.94 | 1.02 | | |
| **Globulin [g/dL],** 2.5-4.0 | T1T2 | 3.33 | 0.39 | | | 3.24 | 0.36 | | | A small significant decrease at T4 of the low dose |
| | T3 | 3.30 | 0.41 | 0.30 | | 3.26 | 0.33 | 0.81 | | but no pattern or significant difference at the high dose |
| | T4 | 3.02 | 0.41 | 8.45 | **<0.025** | 3.18 | 0.38 | 0.14 | | Therefore, no significance. No safety concern. |

CONFIDENTIAL

## Summary of statistical analysis: Aquasearch safety study. Report # RD.0100.001

Summary probabilities: 2-way ANOVA, proportional replication, for two comparisons: T1T2vsT4 AND T1T2vsT3, at each dose.

| PARAMETER | | LOW DOSE: avg | std | F(a/int) | p | HIGH DOSE: avg | std | F(a/int) | p | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Glucose [mg/dL]**, 70-110 | **T1T2** | 92.6 | 13.5 | | | 96.4 | 9.7 | | | |
| | **T3** | 94.7 | 5.8 | 0.60 | | 97.4 | 7.5 | 0.26 | | |
| | **T4** | 91.6 | 9.5 | 0.13 | | 94.1 | 8.4 | 1.43 | | |
| **LDH (lactate dehydrogenase) [IU/L]**, 94-250 | **T1T2** | 170.0 | 33.8 | | | 156.9 | 27.7 | | | A small significant decrease at T4, at both doses, but |
| | **T3** | 162.9 | 24.78407 | 2.55 | | 152.6 | 17.8 | 1.26 | | still within "normal" range. An increase outside of range |
| | **T4** | 159.8 | 23.5 | 8.01 | **<0.025** | 150.6 | 25.3 | 5.29 | **<0.05** | would have raised concern. Not the case, therefore, no safety concern. |
| **Total protein [g/dL]**, 5.9-8.4 | **T1T2** | 7.48 | 0.44 | | | 7.40 | 0.51 | | | A small significant decrease at T4, at both doses, but |
| | **T3** | 7.39 | 0.57 | 0.91 | | 7.38 | 0.50 | 0.12 | | still within "normal" range. No safety concern. |
| | **T4** | 7.27 | 0.52 | 9.39 | **<0.01** | 7.26 | 0.55 | 4.58 | **<0.05** | |
| **WBC (white blood count) [10^9/L]**, 3.5-10.0 | **T1T2** | 6.33 | 1.97 | | | 5.50 | 1.17 | | | |
| | **T3** | 6.04 | 2.04 | 0.60 | | 5.67 | 1.22 | 0.77 | | |
| | **T4** | 6.11 | 1.79 | 0.33 | | 5.61 | 1.40 | 0.26 | | |
| **RBC (red blood count) [10^12/L]**, 3.9-5.2 | **T1T2** | 4.56 | 0.44 | | | 4.57 | 0.41 | | | |
| | **T3** | 4.59 | 0.51 | 0.47 | | 4.58 | 0.42 | 0.03 | | |
| | **T4** | 4.59 | 0.51 | 0.47 | | 4.65 | 0.33 | 3.44 | | |
| **Hemoglobin [g/dL]**, 12-16 | **T1T2** | 14.23 | 1.15 | | | 14.71 | 1.34 | | | |
| | **T3** | 14.11 | 1.23 | 1.23 | | 14.54 | 1.33 | 2.94 | | |
| | **T4** | 14.08 | 1.35 | 1.66 | | 14.68 | 1.08 | 0.05 | | |
| **Hematocrit [%]**, 35-46 | **T1T2** | 41.38 | 3.21 | | | 42.56 | 3.78 | | | A small significant increase at T4, within "normal" range, |
| | **T3** | 41.39 | 3.50 | 0.00 | | 42.58 | 3.76 | 0.00 | | within variability of the method and quite lower than STD |
| | **T4** | 41.83 | 4.12 | 1.05 | | 43.46 | 3.13 | 4.72 | **<0.05** | No significance, no safety concern. |
| **MCV (mean corpuscular volume) [fL]**, 80-100 | **T1T2** | 90.94 | 3.51 | | | 93.11 | 4.39 | | | Same comment as above. |
| | **T3** | 90.86 | 3.39 | 0.33 | | 93.04 | 4.24 | 0.10596 | | |
| | **T4** | 91.22 | 3.21 | 2.14 | | 93.55 | 4.28 | 7.61 | **<0.025** | |
| **MCH (mean corpuscular hemoglobin) [pg]**, 27-33 | **T1T2** | 31.30 | 1.39 | | | 32.19 | 1.52 | | | Small decrease at T3 & T4 of high dose and T4 of low dose. |
| | **T3** | 30.99 | 1.21 | 4.50 | | 31.78 | 1.55 | 14.31 | **<0.0025** | Same comment as for MCV. |
| | **T4** | 30.71 | 1.56 | 13.35 | **<0.0025** | 31.62 | 1.56 | 17.36 | **<0.001** | No safety concern. |
| **MCHC (mean corpuscular hemoglobin concentration) [g/dL]**, 32-36 | **T1T2** | 34.39 | 0.61 | | | 34.55 | 0.45 | | | Same comment as for MCH. |
| | **T3** | 34.07 | 0.63 | 5.93 | **<0.05** | 34.14 | 0.74 | 8.36 | **<0.025** | |
| | **T4** | 33.63 | 0.80 | 18.41 | **<0.001** | 33.80 | 0.76 | 21.20 | **<0.0005** | |
| **RDW (red cell distribution width) [%]**, 11-15 | **T1T2** | 12.87 | 0.73 | | | 12.84 | 0.56 | | | Small significant decrease at T3 of high dose. |
| | **T3** | 12.80 | 0.68 | 0.56 | | 12.68 | 0.51 | 4.63 | **<0.05** | Same comment as for MCHC. |
| | **T4** | 12.65 | 0.51 | 2.25 | | 12.62 | 0.46 | 3.88 | | |
| **Platelet count [10^9/L]** 150-450 | **T1T2** | 262.9 | 69.63 | | | 238.6 | 52.14 | | | Small significant increase at T4 of low dose, but no significant |
| | **T3** | 262.5 | 66.65 | 0.01 | | 242.53 | 50.68 | 0.71 | | increase at T3, or at T3 and T4 of high does. |
| | **T4** | 278.9 | 81.43 | 9.21 | **<0.01** | 247.41 | 55.74 | 3.15 | | Within "normal" range. No safety concern. |
| **Neutrophils [%], 40-70** | **T1T2** | 58.25 | 8.19 | | | 55.24 | 6.77 | | | No pattern. Within "normal" range. |
| | **T3** | 56.75 | 10.30 | 0.74 | | 57.35 | 7.70 | 5.43 | **<0.05** | No significance, no safety concern. |
| | **T4** | 57.88 | 8.35 | 0.03 | | 54.24 | 9.07 | 0.95 | | |
| **Lymphs [%], 20-45** | **T1T2** | 30.75 | 6.97 | | | 32.88 | 5.67 | | | No pattern. Within "normal" range. |

## Summary of statistical analysis: Aquasearch safety study. Report # RD.0100.001

Summary probabilities: 2-way ANOVA, proportional replication, for two comparisons: T1T2vsT4 AND T1T2vsT3, at each dose.

| PARAMETER | | LOW DOSE: avg | std | F(a/int) | p | HIGH DOSE: avg | std | F(a/int) | p | |
|---|---|---|---|---|---|---|---|---|---|---|
| | T3 | 32.00 | 8.85 | 0.56 | | 30.71 | 5.89 | 9.08 | **<0.01** | No significance, no safety concern. |
| | T4 | 31.38 | 6.77 | 0.15 | | 33.94 | 8.37 | 1.04 | | |
| **Monocytes [%], 4-10** | T1T2 | 6.88 | 1.56 | | | 7.82 | 2.05 | | | |
| | T3 | 6.81 | 1.64 | 0.09 | | 8.18 | 2.27 | 1.36 | | |
| | T4 | 7.00 | 1.71 | 0.09 | | 7.94 | 2.11 | 0.25 | | |
| **Eosinophils [%],** 0-6 | T1T2 | 3.22 | 2.28 | | | 3.09 | 1.93 | | | No pattern. Within "normal" range. |
| | T3 | 3.63 | 2.66 | 7.75 | **<0.025** | 2.94 | 1.98 | 0.80 | | No significance, no safety concern. |
| | T4 | 2.94 | 2.11 | 0.97 | | 3.06 | 1.85 | 0.02 | | |
| **Basophils [%],** 0-2 | T1T2 | 1.00 | 0.37 | | | 0.91 | 0.38 | | | |
| | T3 | 0.81 | 0.54 | 0.79 | | 0.82 | 0.53 | 0.81 | | |
| | T4 | 0.81 | 0.54 | 0.79 | | 0.82 | 0.53 | 0.38 | | |
| **PTT (activated partial** | T1T2 | 29.63 | 2.38 | | | 29.72 | 2.21 | | | Small significant increase at T3 & T4 of high dose, within |
| **thromboplastin time) [sec],** 27-35 | T3 | 29.80 | 2.33 | 0.34 | | 30.42 | 1.83 | 6.19 | **<0.025** | "normal" range, and less than STD. Also no change at |
| | T4 | 29.36 | 1.79 | 0.25 | | 31.15 | 2.30 | 7.32 | **<0.025** | the low dose. No safety concern. |
| **Urine specific gravity [g/L]** | T1T2 | 1.020 | 0.006 | | | 1.02 | 0.01 | | | |
| 1.005-1.03 | T3 | 1.020 | 0.007 | 0.09 | | 1.02 | 0.01 | 0.90 | | Very minor change at T4 of low dose. No change at high dose |
| | T4 | 1.016 | 0.006 | 5.91 | **<0.05** | 1.02 | 0.01 | 3.82 | | No significance, no safety concern. |
| **Urine pH,** 5.0-7.5 | T1T2 | 5.77 | 0.85 | | | 5.66 | 1.55 | | | |
| | T3 | 5.78 | 0.91 | 0.00 | | 5.80 | 1.53 | | | |
| | T4 | 5.41 | 0.69 | 4.00 | | 5.72 | 0.84 | | | |

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington DC 20205

Aquasearch Inc.
bringing the oceans to life

Aquasearch Inc.
Kona Production Facility
73-4460 Queen Kaahumanu Highway
Suite 110, Kailua-Kona, HI 96740 USA
Tel: (808)326-9301 Fax: (808)326-9401
WebSite: http://www.aquasearch.com/
E-mail: aqse@aquasearch.com

December 6, 1999

Re: New Dietary Ingredient Notification for *Haematococcus* algal meal. Rat safety study.

Dear Administrator,

As part of our New Dietary Ingredient Notification for *Haematococcus* algal meal, MBR Laboratories have completed a rat toxicity study. Their findings summarized in their preliminary report, enclosed (section 5), confirm the safety and absence of sub-chronic toxicity of the algal meal at the doses tested. A final report will be available shortly. We will send you a copy before the end of the 75-day period after notification.

Thank you very much in advance for the attention that you will give to our notification. If you have any question, do not hesitate contacting us at (808)326-9301.

Sincerely yours,

Martin Guérin
V.P. Marketing & Sales
Aquasearch Inc.

000079

**MB RESEARCH LABORATORIES**
1765 Wentz Road
P.O. Box 178
Spinnerstown, PA 18968

# FAX COVER SHEET

| | | | |
|---|---|---|---|
| DATE: | November 19, 1999 | TIME: | 12:54 PM |
| TO: | Martin Guerin<br>Aquasearch, Inc. | PHONE:<br>FAX: | 808-326-9301<br>808-326-9401 |
| FROM: | Edward Yurkow, Ph.D.<br>MB Research | PHONE:<br>FAX: | 215-536-4110<br>215-536-1816 |
| RE: | Preliminary Report: 28 DAY REPEATED DOSE ORAL TOXICITY STUDY IN RATS | | |
| CC: | Dr. Shayne Gad (FAX. 919-632-5877) | | |

Number of pages including cover sheet: 2

## *Message*

Study Details: The 28 day repeated oral dose toxicity study in rats conducted using a test article (*Haematococcus pluvialis*) supplied by Aquasearch, Inc., was initiated on Thursday, October 7th and was terminated on Thursday, November 4th, 1999. The test article (a dark red powder) was administered orally to the rats using corn oil as vehicle.

Summary of Study Design: In this study, three (3) groups of 20 rats each (10 M/10F) were dosed by gavage for 28 consecutive days. One set of animals was a high dose group receiving 50 mg/kg/day while the low dose group received 5 mg/kg/day. A control group (20 rats) received the vehicle (corn oil) alone. The rats were observed daily (body weights were determined weekly) and on the 29$^{th}$ day of the study, the animals were sacrificed.

Status of Study: A necropsy was conduced and tissues were preserved for histopathological examination (in-progress). Hematology and clinical chemistry has been recently completed and the data is currently being analyzed. Analysis of the data should be completed within 4-6 weeks.

Preliminary Observations:

High Dose Group: None of the 20 rats in the high dose group died or exhibited any overt signs of toxicity during the in-life phase of this study. A moderate aversion to delivery of the test article was observed for three days in the first week of the study but subsided and was not evident throughout the rest of the study. At necropsy, all

major organs of the animals in dosage group appeared normal. Body and organ weights are currently being analyzed.

Low Dose Group: Two rats in this dose group died during the in-life phase of the study. The cause of death for both rats is likely to be the aspiration of the test article resulting in pulmonary distress/failure. Signs of pulmonary distress were noted immediately following dosing and the rats died within 12 hour of dosing. Necropsy was conducted on the rats and tissues were preserved for histopathological examination. Consistent with the suspected cause of death was the finding of fluid in the plural cavity of both rats. In addition, the lungs appeared to be stained with the orange-red (colored) test article. The results of the histological evaluation will be used to assess whether or not these deaths were due to aspiration of the test article. None of the remaining 18 rats in this group died or exhibited any overt signs of toxicity during the in-life phase of this study. Since these deaths occurred in the low dose group and no deaths were observed in the high dose group, this lack of a dose response indicates that deaths were not due to the test article.

Control Group: None of the 20 rats in the control group died. A technician observed difficulty during the administration of the vehicle to one specific rat for several days in the last week of the study. The gavage needle appeared to encounter an obstruction during administration of the test article. The reason for this difficulty was not obvious. At necropsy, the esophagus and stomach of this rat was inspected visually and no obvious reasons for the obstruction were evident, however, the esophagus appeared slightly thickened. The esophagus from this rat was excised and placed in fixative for pathological examination.

Conclusions: Based on a preliminary review of the data, the test article administration did not cause mortality or an increase in adverse system observations when compared to the control group. Further analysis of the data (histopathology/ nematology/clinical chemistry) will more fully characterize any adverse effect on the health of rats.

It must be noted that the above represents a preliminary report based on a cursory review of the data that have been generated during the study. Individual and group statistics, data analysis, histological evaluation as well as results of hematology/ clinical chemistry are currently being performed. Results of this study will be completely described in the Final Report. If you have any questions concerning aspects of this study please call. Thank you.

Regards

Edward J. Yurkow

Edward J. Yurkow, Ph.D.
Director of Research

000072

T:\YURKOW\AquaSearch\AsPrlmRpt.doc

Nov 19 99 02:05p MB Research (215)536-1816 p.2

# MB RESEARCH LABORATORIES

# AQUASEARCH PROTOCOL

**1.0 TITLE OF STUDY: 28 DAY REPEATED DOSE ORAL TOXICITY STUDY IN RATS**

**2.0 OBJECTIVE**: To provide information on the possible health hazards likely to arise from repeated oral administration of the test article during a 28 day period.

**3.0 TEST ARTICLE:**

3.1: Source: All test articles will be supplied by the sponsor. Prior to the initiation of the study, there should be a characterization of the test substance, including purity of the test article and if technically feasible, the name and quantities of unknown contaminants and impurities. Analyses of test article is the responsibility of the sponsor.

3.2: Label: Each test article will be identified by source, name and/or code number, date of receipt at MB Research, and MB Project Number.

3.3: Storage: The test and control substances will be stored at room temperature and humidity.

3.4: Hazards: Based on the information provided by the sponsor, appropriate routine safety precautions will be exercised in the handling of the test article.

3.5: Vehicle: The vehicle will be corn oil.

**4.0 GENERAL TEST SYSTEM PARAMETERS:**

4.1: Animal Requirements:

| | | |
|---|---|---|
| 4.1.1: Number of Animals in Quarantine | : | 70+ |
| 4.1.2: Number of Animals on Study | : | 60 |
| 4.1.3: Number of Groups | : | 2 Test Article Groups & Vehicle Control Group |
| 4.1.4: No. Animals/Group | : | 20 |
| 4.1.5: Sex | : | Equal #'s Male & Female (nulliparous & non-pregnant) |
| 4.1.6: Species/Strain | : | Rat/Wistar Albino |
| 4.1.7: Age @ study initiation | : | less than 9 weeks |

4.2: Justification for Species and Number of Animals:

4.2.1: Species: The rat is the system of choice because it has been shown to be sensitive to toxic effects of a variety of chemicals, is a standard animal model for the sub-chronic oral toxicity test, and is the preferred species in the regulatory requirements.

4.2.2: Number of Animals: Twenty animals/group is the number required in order to provide sufficient data for analysis.

4.3: Husbandry:

4.3.1: Housing: Animals will be housed 1/cage in suspended cages which conform to the size recommendations in Guide for the Care and Use of Laboratory Animals DHEW (NIH). ANIPADS™, placed beneath the cage, will be changed at least three times/week. Feed containers will be changed and sanitized every week. The animal room, reserved exclusively for rats, is temperature controlled, and is equipped with a 12-hour light/dark cycle. Temperature and humidity will be continuously recorded using automatic recording devices.

4.3.2: Equilibration: The test animals will be conditioned to the housing facilities for at least 5 days prior to testing.

4.3.2.1: Equilibration Observations: Animals will be observed once daily for general health. Body weights will be taken and recorded immediately prior to study initiation.

4.3.3: Food: Certified Purina Rodent Chow (Diet #5002) is available ad libitum. The certified analysis of the rodent chow will be included in the study file and each lot utilized will be identified and recorded in the study file.

4.3.4: Water: Water will be available ad libitum.

4.3.4.1: Analysis of Water and Acceptable Levels of Contaminants: Analysis of water is performed at periodic intervals as defined in MB's SOPs and results are compared against a list of acceptable contaminants as provided by the water testing laboratory.

4.4: Control of Bias: From the available pool of animals, free from any evidence of disease or abnormality, and of the sex and weight range specified herein, rats will be selected and assigned to groups using a computer program which generates random numbers. At study initiation, the weight variation of each animal will be within twenty percent (20%) of the mean body weight for each sex.

4.5: Identification:

4.5.1: Cage: Each cage is identified by a cage tag indicating the test article identification, MB project number, dose level, number and sex of animals.

4.5.2: Animal: Each animal is identified by a uniquely numbered metal eartag.

## 5.0 EXPERIMENTAL DESIGN:

5.1: Route of Administration: The test article or vehicle will be administered orally by gavage on a mg/kg of body weight basis.

5.1.1: Justification for Route of Administration: The oral route of administration is chosen because it is the intended route of human exposure.

5.2: Dose Levels: Based on available toxicity data and in consultation with the sponsor, at least 2 dose levels and a vehicle control group will be used.

5.3: Frequency: Once daily, seven (7) days per week for 28 consecutive days.

5.4: Dosing Schedule:

| GROUP | DOSE (mg/kg) | # OF ANIMALS |
|---|---|---|
| Vehicle Control | -0- | 10 Male & 10 Female |
| Test Article-High Dose | 50 mg/kg | 10 Male & 10 Female |
| Test Article-Low Dose | 5 mg/kg | 10 Male & 10 Female |

## 6.0 DOSING PROCEDURE:

Amendment #1 - section 6.1 replaced.

6.1: Sample Preparation: Dosing solutions will be prepared weekly by dissolving/suspending the test article in corn oil to a concentration of 5 mg/ml (for low dose group) and 50 mg/ml (for high dose group). The dosing solutions and vehicle will be administered on a 1 ml/kg basis such that the low dose and high dose rats receive 5 mg/kg and 50 mg/kg of the test article, respectively.

6.1.1: Reserve Samples from each batch of the test or control article will be retained and forwarded to the sponsor upon submission of the report.

6.2: Sample Description: The observable physical properties of the test article are recorded.

6.3: Treatment: The test article or vehicle will be measured by syringe and dosed via syringe and a dosing needle. Individual dose volumes will be recorded. The dose will be calculated on a mg/kg of body weight basis and adjusted weekly. Animals will be dosed at similar times each day. The maximum volume of liquid administered at one time will not exceed 1.0 ml/100 g of body weight.

## 7.0 TYPE & FREQUENCY OF OBSERVATIONS:

7.1: In Vivo:

7.1.1: Systemic Observations: Animals will be observed once daily for toxicity and pharmacological effects and twice daily (a.m. and p.m.) for morbidity and mortality. Moribund animals will be humanely sacrificed.

7.1.2: Body Weights will be recorded immediately pretest, weekly, at death, and at termination in the survivors.

7.1.3: Food Consumption: The amount of food consumed will be measured during the fourth week of the study.

7.1.4: Clinical Pathology at Study Term: All survivors will be fasted overnight prior to scheduled termination sacrifice. On the day of sacrifice, animals will be anesthetized with ether and blood will be drawn from the dorsal aorta.

All slides and refrigerated whole blood will be shipped with ice packs, ~~frozen plasma and~~ frozen serum will be shipped on dry ice, for overnight delivery to Ani Lytics, Inc., Gaithersburg, MD for analyses as indicated below. Refer to the study file for specimen collection and shipment instructions.

7.1.4.1: Hematology will be performed on 5 male and 5 female randomly selected from each group. Blood will be drawn into lavender-top tubes containing EDTA. The tubes will be immediately mixed by repeated inversions and then refrigerated. Blood smears will be prepared with the blood from these tubes. Whole blood will be analyzed for:

red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, white blood cell count, differential leukocyte count, and platelet count.

7.1.4.2: Clinical Chemistry will be performed on all animals. Blood will be collected into a plain red top tube or serum separator tube. The blood will be allowed to clot at room temperature for at least 15 minutes and then centrifuged. The serum will be separated, frozen and will be shipped on dry ice. Serum will be analyzed for:

| | | |
|---|---|---|
| Magnesium | Aspartate aminotransferase | Alkaline Phosphatase |
| Sodium | Albumin | Gamma Glutamyl transpeptidase |
| Alanine Aminotransferase | Globulin | Creatinine |
| Urea nitrogen | Total cholesterol | Glucose (fasting) |
| Total bilirubin | Triglycerides | Total protein |
| Calcium | Chloride | Creatine Kinase (CPK) |
| Phosphorous | Potassium | |

7.1.4.3: Urinalysis will be performed during the last week of the study using timed urine volume collection. The volume of urine collected will be recorded and included in the study report. An aliquot of the urine will be sent for analysis, and determinations will include: appearance, osmolality or specific gravity, pH, protein, glucose and blood/blood cells.

7.2 Post Mortem:

7.2.1: Spontaneous Deaths will be recorded, necropsied and tissues preserved.

7.2.2: Moribund Animals will be humanely sacrificed using ether and exsanguination and necropsied as soon as possible to prevent loss of tissues through autolysis.

7.2.3: Termination of Survivors: On day 29, survivors will be humanely sacrificed using ether and exsanguination.

7.2.4: Necropsy: On day 29, all survivors will be subjected to gross necropsy which will include examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

7.2.5: Organ Weights: The liver, spleen, heart, testes/ovaries, kidneys and brain from all animals will be trimmed and weighed wet as soon as possible after dissection.

7.2.6: Tissues:

7.2.6.1: Frozen Tissues: The following solid tissues from all animals will be excised, rinsed with sterile saline to remove extraneous tissues/excess blood, wrapped closely with aluminum foil and each package labeled with MB Project number, tissue type, animal number and sex, test article, group #, dose level and date. Packages will be immediately frozen on dry ice and shipped on dry ice to sponsor's designee. If tissues are not shipped on the day of necropsy, they will be stored frozen at -20°C.

| | |
|---|---|
| Liver (section) | Blood plasma (2 ml/animal) |
| Skin (1" square from belly area) | Brain (left side) |
| Fat (from belly area) | Quadriceps (left) |
| Eye (1)(left) | |

see amendment #2 Sections 7.2.6.1 & 7.2.6.2 replaced

7.2.6.2: Neutral Buffered Formalin: The following tissues and organs from all animals will be preserved in 10% buffered formalin:

| | |
|---|---|
| Liver (part) | Eye (1)(right) |
| Spleen | Kidneys |
| Heart | Thyroid |
| Brain (right side) | Bone marrow |
| Skin (1" square from belly area) | Thymus |
| Testes/Ovaries | |

7.2.7: Histopathology: All tissues listed in section 7.2.6.2 from animals in the high dose group and in the control group will be examined microscopically. If necessary, tissues from other dose groups will be examined at the option of the sponsor. Histopathology examinations will be performed by W. Ray Brown, D.V.M., Ph.D., Research Pathology Services, Inc., New Britain, PA.

8.0 TEST DURATION: The duration of this study is 29 days. However, at the sponsor's option, the study may be extended beyond the 29 day period. Any extension will be at additional cost.

9.0 ANALYSIS OF DATA:

9.1: Statistical Calculations:

9.1.1: Non-discrete Data including clinical chemistry, hematology, organ weights, body weights, organ/body weight ratios and food consumption will be tabulated and means and standard deviations calculated. In addition, an Analysis of Variance (ANOVA) will be performed on absolute organ and body weights, and on organ/body weight ratios. For parametric data, Tukey-Kramer post hoc tests will be performed. For non-parametric data, Kruskal-Wallis analysis of variance with Dunn's post hoc test will be done.

9.1.2: Discrete Data Mortality and other discrete data will be analyzed by Fisher's Exact Test and/or be presented in tabular form.

9.2: Evaluation of Results: The evaluation will include the relationship between the dose of the test article and the presence or absence, the incidence and severity of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects.

10.0 REVISION OF THE PROTOCOL:

Any amendment to or deviation from this protocol will be fully documented in the study file, including the reason for the change, the authority for said change and the date thereof.

11.0 RECORDS TO BE MAINTAINED:

11.1: Collection of Data: All data generated during the conduct of this study will be recorded in ink on worksheets. All entries will be dated, initialed and verified by another person.

11.2: Reports:

11.2.1: Draft Report: A draft report will be submitted prior to submission of the final report.

11.2.2: Final Report: Following approval by the sponsor of the draft report, the final report will be submitted and will include, but not be limited to, test article characterization, identification and composition, if available, test system identification, test procedures and test results including tabulated data of individual toxic responses, body weight data, food consumption, hematology and clinical chemistry evaluations, necropsy findings, histopathological findings and statistical treatment of the results.

11.3: Retention of Data: All data generated as a result of the conduct of this study will be retained in the archives at MB Research for an indefinite period of time, but not less than 10 years from the date of the final report of this study. Prior to disposing of any of the records generated from the conduct of this study, the sponsor will be notified in writing.

11.3.1: Raw Data will be filed at MB Research by project number.

11.3.2: Final Reports will be filed at MB Research by sponsor name and MB project number.

11.3.3: Test Article: Any remaining test article will be returned to the sponsor upon submission of the report.

11.3.4: Tissues, blocks and slides will be stored at MB Research by sponsor name and MB project number for one year following submission of the final report. After one year, the sponsor will be contacted to determine final disposition.

## 12.0 GOOD LABORATORY PRACTICES:

This study will be conducted in accordance with the Good Laboratory Practices Regulations of the EPA 40 CFR 160 and 792, the FDA 21 CFR 58, and the Organization for Economic Cooperation & Development (OECD), The Testing of Chemicals, 1997[1].

12.1: Protocol: MB Research will have on file a copy of this protocol, signed and dated by both the responsible MB Study Director and the sponsor's authorized representative.

12.2: Quality Assurance: The Quality Assurance Unit will inspect at least three (3) in-life phases of this study, audit the raw data and audit the report in accordance with the Standard Operating Procedures (SOP's) of MB and the applicable government regulations.

Received 26 Oct 9

[1]10/05/99: page reprinted to correct date of OECD GLPs (Section 12.0).

## 13.0 SPONSOR REQUEST:

13.1: The sponsor requests that this protocol be implemented:

☐ As written (*or*) ☐ Amended per attached description of amendments

13.2: Test Article: The test article will be identified in the report and supporting documentation exactly as indicated by the sponsor in section 13.2.1.

13.2.1: Identity: The test article is identified as follows: _____

_____

pH (when applicable): _____ Lot/Batch #: _____

13.2.2: Analysis of aliquots of dosing mixture(s) required? ☐ yes ☐ no Frequency: _____

13.2.3: Test Article Characterization including identity, strength, stability, solubility and purity is routinely required by regulatory agencies in support of data submissions (EPA 40 CFR 160.105 and 792.105; FDA 21 CFR 58.105, OECD 2.3 -Test and Reference Substances). This information is:

☐ Attached ☐ Filed with sponsor ☐ Study will not be submitted to a regulatory agency.

13.2.4: Material Safety Data Sheet Supplied: ☐ Yes ☐ No

13.2.5: Estimated Date of Arrival @ MB Research: _____

13.3: Authorization Statement: This protocol is authorized for implementation at MB Research. This study is necessary to estimate the toxic effects of the test compound. To the best of my knowledge and information, this test is not an unnecessary duplication of any previous studies.

13.3.1: Confidentiality: Study results and reports will be released only to the below named sponsor representative unless other sponsor representatives are identified herein.

BY: _____ (signature) (date)

_____ (typed name)

_____ (title)

FOR: _____ (company Name)

_____ (address)

_____ (city) (state) (zip)

_____ (phone) (fax)

Other Sponsor Representative: _____

14.0 MB RESEARCH ACKNOWLEDGMENT: Request for implementation of this protocol and receipt of the test article is acknowledged by MB Research.

14.1 Test Article Identity: _____

_____

14.1.1: Date Received: _____

14.1.2: Physical Description: _____

14.2: MB Project Number assigned to this study: _____

14.3: Animal Supplier: The Licensed U.S.D.A. animal supplier is: _____

14.4: Proposed Study Dates:

14.4.1: Experimental Start Date: _____

14.4.2: Experimental Term Date: _____

14.4.3: Study Completion Date (Submission of Report): Approximately 3 months following Experimental Term Date.

14.5: Approval: There are currently no suitable non-animal alternatives to this study as determined according to MB Research SOP Vol. III A.6. This protocol is designed to avoid or minimize discomfort. The procedures will be performed by personnel thoroughly trained in the humane care and use of laboratory animals. If pain does occur as a result of the nature of the test article being used, it will be addressed according to MB SOP Vol. III.A.2.f. This protocol is approved for implementation at MB Research by the below named MB Study Director.

_____

BY: Study Director (date)
MB Research Laboratories

This protocol was originally reviewed by the Institutional Animal Care and Use Committee (IACUC) of MB Research on the date indicated below and found to be in compliance with acceptable standards of animal welfare and humane care. The IACUC committee will review this protocol on an annual basis. This review will be documented in the IACUC minutes and included in the semi-annual report to the institutional official.

DATE: 3/31/98

# PROTOCOL AMENDMENT

DATE OF AMENDMENT : 10/05/99
PROTOCOL TITLE : 28 Day Repeated Dose Oral Toxicity in Rats
PROTOCOL # : Client Protocol
MB PROJECT # : MB 99-7853.01
SPONSOR : Aquasearch, Inc.
AMENDMENT # : 1

**Description of Amendment:**

6.0 DOSING PROCEDURE: *Replace entire section 6.0 with the following*

6.0 DOSING PROCEDURE:

6.1 Sample Preparation:

6.1.1 Preparation of LOW DOSE solution (0.25% solution of TA) for delivering 5 mg TA/kg: 281.25 grams of Mazola corn oil (specific gravity of batch used was determined to be 0.94 g/ml) will be weighed out into a 500 ml glass beaker. 0.75 grams of Aquasearch TA will then be added to the beaker containing the corn oil. The total volume of the preparation will be verified and brought to 300 ml with corn oil if necessary (see Dr. Yurkow). A clean stir bar will be placed into the beaker and the contents will be stirred for at least 15 minutes at room temperature. The resulting preparation should appear dark red/orange in color with undissolved material evenly suspended in the oil. Dosing solutions will be prepared on a weekly basis.

6.1.2 Preparation of HIGH DOSE solution (2.5% solution of TA) for delivering 50 mg TA/kg: 274.50 grams of Mazola corn oil (specific gravity of batch used was determined to be 0.94 g/ml) will be weighed out into a 500 ml glass beaker. 7.5 grams of Aquasearch TA will then be added to the beaker containing the corn oil. The total volume of the preparation will be verified and brought to 300 ml with corn oil if necessary (see Dr. Yurkow). A clean stir bar will be placed into the beaker and the contents will be stirred for at least 15 minutes at room temperature. The resulting preparation should appear dark red/orange in color with undissolved material evenly suspended in the oil. Dosing solutions will be prepared on a weekly basis.

6.1.3 Reserve Samples: Reserve samples of the corn oil (20 ml) and the test article (10 g) will be taken and frozen prior to study initiation. On day 1 and day 7, aliquots (10 ml) will be taken from the top, middle and bottom of the preparation while it is being stirred for dosing. The samples will be labeled, stored frozen for possible future analysis.

6.1.4 Storage of TA: Following preparation or use of the TA for dosing, the tops of the beakers will be covered with plastic wrap and then the beaker will be wrapped with aluminum foil to protect it from light. The TA in corn oil will be stored at 4°C.

6.1.5 Daily Sample Procedures: The beakers containing the TA will be removed from the refrigerator and stirred on a stir plate while allow to warm to room temperature for at least 15 minutes.

6 2: Sample Description: The observable physical properties of the test article will be recorded.

6 3: Treatment: Body weights will be taken and recorded immediately prior to study initiation and on a weekly basis. The most recent body weight will be used for dosing. Gavage syringes will be filled with the suspended TA while the suspension is being stirred. The TA, suspended in corn oil, will be dosed on a ml per kg body weight basis. For both dosage groups, the dose volume is 2 ml/kg. For example, a rat weighing 200 grams (0.200 kg) would receive 0 40 ml of the suspended TA

**Reason for Amendment:**

To more fully describe sample preparation procedures.

## AMENDMENT APPROVED BY:

FOR: MB RESEARCH LABORATORIES
P. O. Box 178
Spinnerstown PA 18968

Edward Yurkow 10/5/99
Edward Yurkow, Ph.D. date
Study Director

FOR: AQUASEARCH, INC.
73-4460 Queen Kaahumanu Hwy.
Kailua-Kona, HI 96740

Mia D'Unson 5Oct1999
Mia Unson, Ph.D. date
Research Scientist, Sponsor Representative

# PROTOCOL AMENDMENT

| | | |
|---|---|---|
| **DATE OF AMENDMENT** | : | **10/25/99** |
| **PROTOCOL TITLE** | : | **28 Day Repeated Dose Oral Toxicity In Rats** |
| **PROTOCOL #** | : | **Client Protocol** |
| **MB PROJECT #** | : | **99-7853.01** |
| **SPONSOR** | : | **AQUASEARCH, INC.** |
| **AMENDMENT #** | : | **2** |

*Replace sections 7.2.6.1 and 7.2.6.2 with the following:*

7.2.6.1: Frozen Tissues: The following solid tissues from all animals will be excised, rinsed with sterile saline to remove extraneous tissues/excess blood and wrapped closely in a piece of aluminum foil that has been labeled with the MB Project Number, tissue type, animal number and sex, test article, group #, dose level and date. Packages will be immediately frozen on dry ice. All (individually wrapped) frozen samples from a single rat will be placed into a separate plastic bag that has been labeled with all pertinent information concerning the specific rat. Samples will be stored frozen (-20°C) and shipped on dry ice to sponsor's designee. If tissues are not shipped on the day of necropsy, they will be stored frozen at -20°C.

| | |
|---|---|
| Liver (at least 1 gram) | Left Side of Brain |
| Skin (1 inch square from belly area) | Left Eye |
| Quadriceps Muscle (Left) | Blood plasma (2 ml/animal) |

7.2.6.2: Neutral Buffered Formalin: The following tissues and organs from all animals will be preserved in 10% neutral buffered formalin:

| | |
|---|---|
| Liver (part) | Right Side of Brain |
| Spleen | Right Eye |
| Heart | Thyroid |
| Skin (1 inch square from belly area) | Bone marrow |
| Kidneys | Thymus |
| Testes/Ovaries E.A.Y 11/1/99 | |

To more specifically describe the organs and tissues to be isolated and processed and to amend the tissues that are to be frozen.

**AMENDMENT APPROVED BY:**

**FOR: MB RESEARCH LABORATORIES**
1765 Wentz Road, P.O.Box 178
Spinnerstown, PA 18968

Edward A. Yurkow 10/28/99
Edward J. Yurkow, Ph.D date
Study Director

**FOR: AQUASEARCH, INC.**
73-4460 Queen Kaahumanu Hwy., Suite 110
Kailua-Kona, HI 96740

10/28/99
Mike Unson, Research Scientist date
On behalf of Dr. Unson,
Martin Guerin, VP. Marketing & Sales



n:\protocolsamendmen\7853-amd.2

000084

10/28/1999 18:42 8083269401 AQUASEARCH, INC. PAGE 02
Oct 28 99 03:45p MB Research (215)536-1816 p.2

Aquasearch Inc.
bringing the oceans to life

Aquasearch Inc.
Kona Production Facility
73-4460 Queen Kaahumanu Highway
Suite 110, Kailua-Kona, HI 96740 USA
Tel: (808) 326-9301 Fax: (808) 326-9401
WebSite: http://www.aquasearch.com/
E-mail: aqse@hula.net

## Lot 990610Mix

Lot 990610Mix of *Haematococcus pluvialis* algal meal consists of the harvest from five ponds during 6 – 19 May 1999. The algal mass from an individual pond was stored frozen (7 days) and processed by cell breaking and drying during 13 –26 May 1999 as detailed in the following table. The five dryer batches were stored frozen until ground and mixed into a single lot (990610MIX) on 10 June 1999.

| Lot | Dryer Batch | Pond Harvested | Weight (kg) |
|---|---|---|---|
| 990610MIX | 990513A | P04-990506 | 7.52 |
| | 990518B | P06-990511-2 | 13.4 |
| | 990520A | P04-990513 | 14.2 |
| | 990524A | P05-990517 | 13.52 |
| | 990526A | P03-990519 | 9.28 |

The astaxanthin content of Lot990610Mix was found to be 2.3% by Aquasearch's standard spectrophotometric assay (cf. Attached C.O.A.).

This result was confirmed by subsequent analysis by Akvaforsk (see attached report) who reported 2.18% astaxanthin by HPLC assay.

The small difference between these two values, can be easily explained by the variability of both methods, as well as small differences in methodology: as discussed in TR.1005.001, the extraction process by Aquasearch is more thorough than the one used by Akvaforsk.

This Lot990610Mix is highly representative of Aquasearch product quality. It has been used subsequently in both safety studies conducted by Aquasearch.

Aquasearch Inc.
bringing the oceans to life

Aquasearch Inc.
Kona Production Facility
73-4460 Queen Kaahumanu Highway
Suite 110, Kailua-Kona, HI 96740 USA
Tel: (808) 326-9301 Fax: (808) 326-9401
WebSite: http://www.aquasearch.com/
E-mail: aqse@hula.net

# Certificate of Analysis

| | | |
|---|---|---|
| *Product* | : | Algae meal from the green algae *Haematococcus pluvialis* |
| *Lot number* | : | 990610MIX (***1 x 10 kg***) |
| *Date of manufacture* | : | June 10, 1999 |
| *Date of analysis* | : | August 5, 1999 |
| *Moisture (% of total weight)* | : | 2.3 |
| *Astaxanthin (% of total weight)* | : | 2.3 |
| *Aerobic Plate Count (TVC)* | : | <1000 |
| *Approved by* | : | M. Olaizola, Ph. D. |

000083

Aquasearch Inc.
bringing the oceans to life

Aquasearch Inc.
Kona Production Facility
73-4460 Queen Kaahumanu Highway
Suite 110, Kailua-Kona, HI 96740 USA
Tel: (808) 326-9301 Fax: (808) 326-9401
WebSite: http://www.aquasearch.com/
E-mail: aqse@hula.net

# Certificate of Analysis

| | | |
|---|---|---|
| *Product* | : | Tablets from the green algae *Haematococcus pluvialis* |
| *Lot number* | : | EP 9441 |
| *Date of manufacture* | : | September 10, 1999 |
| *Date of analysis* | : | October 25, 1999 |
| *Astaxanthin (% of total weight)* | : | 0.517 |
| *Weight of tablets* | : | 0.745 g |
| *Astaxanthin per tablet* | : | 3.85 mg |
| *Aerobic Plate Counts (per gram)* | : | <1000 |
| *Approved by* | : | M. Olaizola, Ph. D. |

000087

# ANALYSIS REPORT, AKVAFORSK, N-6600 SUNNDALSØRA, NORWAY

| | |
|---|---|
| ***Projekt :*** | s827 External samples |
| ***Responsible :*** | Bjørn Bjerkeng |
| ***Material :*** | Haematococcus meal, acetone extract , rice bran oil extract from Aquasearch |
| ***Samples:*** | (1) Haematococcus pluvialis acetone extract, ampoule #1198.<br>(2) 990610MIX rice bran oil extract<br>(3) 990610MIX |
| ***Analyses :*** | Carotenoids |
| ***Date of analysis:*** | 29.06.1999, 06.07.1999. |
| ***Signature :*** | KSN |

03000

# Results:

**HPLC**

| Sample | **Total astaxanthin mg/kg** | Diesters mg/kg Rt=1,5 min | Monoesters mg/kg Rt=3,2-4,0 min | All-E astaxanthin mg/kg Rt=10,7 | 9Z-astaxanthin mg/kg Rt=11,8 | 13Z-astaxanthin mg/kg Rt=12,6 | Other isomers mg/kg Rt=13,4 |
|---|---|---|---|---|---|---|---|
| (1) | 73301 | 12091 | 59566 | 931 | 79,7 | 137 | 496 |
| (2) | 16280 | 2484 | 13293 | 308 | 47,6 | 45,2 | 102 |
| (3) | 21753 | 3187 | 17791 | 449 | 80,1 | 84,6 | 160 |

| Sample | **Total astaxanthin mg/g** | Diesters % of total Rt=1,5 min | Monoesters % of total Rt=3,2-4,0 | All-E astaxanthin % of total Rt=10,7 | 9Z-astaxanthin % of total Rt=11,8 | 13Z-astaxanthin % of total Rt=12,6 | Other isomers % of total Rt=13,4 |
|---|---|---|---|---|---|---|---|
| (1) | 73,3 | 16,5 | 81,3 | 1,3 | 0,1 | 0,2 | 0,7 |
| (2) | 16,3 | 15,3 | 81,7 | 1,9 | 0,3 | 0,3 | 0,6 |
| (3) | 21,8 | 14,6 | 81,8 | 2,1 | 0,4 | 0,4 | 0,7 |

**Spectrophotometric**

| Sample | Total carotenoids mg/kg meal | mg/g | % of total mass |
|---|---|---|---|
| (1) | 72073 | 72 | 7,2 |
| (2) | 16734 | 17 | 1,7 |
| (3) | 26344 | 26 | 2,6 |

**Yellow xanthophylls represented approximately 2% of total carotenoids based on chromatogram areas**

Sandaldora 26. Oct., 1999

Bjørn Bjaknes

980000

Warren Analytical Laboratory
P.O. Box G
650 "0" Street
Greeley, Colorado 80632-0350
(970) 351-6344/Fax: (970) 351-6648
1-800-945-6669

**LABORATORY ANALYSIS REPORT**
**TRADE SECRET**
**Confidential Commercial Report**

Attn: MIA UNSON
AQUASEARCH, INC.



Work Order No. 9914382 Flakes

Receive Date: 06/18/99 Reported: 07/13/99

| SMP. DATE | IDENTIFICATION | TYPE | I.D. |
|---|---|---|---|
| 06/16/99 | 990610 MIX H.PLUVIALIS FLAKES | Flakes | 99IR-01900 |

| | | | | | |
|---|---|---|---|---|---|
| Phosphorus | 3900 | ppm | Arsenic | <0.5 | ppm |
| Cadmium | <0.5 | ppm | Cobalt | 0.58 | ppm |
| Chromium | 5.1 | ppm | Mercury | <0.1 | ppm |
| Potassium | 2300 | ppm | Magnesium | 1500 | ppm |
| Molybdenum | <0.5 | ppm | Nickel | 5.7 | ppm |
| Lead | <0.5 | ppm | Selenium | <0.5 | ppm |
| Zinc | 49 | ppm | Niacin | 0.45 | mg/100g |
| Thiamine (B1) | 0.09 | mg/100g | | | |
| Riboflavin (B2) | 0.26 | mg/100g | | | |
| Alpha Tocopherol | 411.8 | mcg/g | | | |
| Gamma Tocopherol | 1.4 | mcg/g | | | |
| Delta Tocopherol | 0.0 | mcg/g | | | |

**David Jankow**
**Laboratory Director**

Warren Analytical Laboratory
P.O. Box G
650 "O" Street
Greeley, Colorado 80632-0350
(970) 351-6344/Fax: (970) 351-6648
1-800-945-6669

# LABORATORY ANALYSIS REPORT
## TRADE SECRET
## Confidential Commercial Report

Attn: MIA UNSON
AQUASEARCH, INC.



Work Order No. 9914380 Flakes

Receive Date: 06/18/99 Reported: 06/18/99

| SMP. DATE | IDENTIFICATION | TYPE | I.D. |
|---|---|---|---|
| 06/16/99 | 990610 MIX H.PLUVIALIS FLAKES | Flakes | 99NL-00255 |

| | | | | | |
|---|---|---|---|---|---|
| Crude Fiber | 4.30 | g | Peroxide | N.D. | meq/kg |
| Free Fatty Acid | N.D. | g | Acid Hydrolysis Fat | 19.43 | g |
| Ash | 3.28 | g | C-8:0 | <0.01 | g |
| C-10:0 | <0.01 | g | C-12:0 | <0.01 | g |
| C-14:0 | 0.09 | g | C-14:1 | <0.01 | g |
| C-16:0 | 2.82 | g | C-16:1 | 0.11 | g |
| C-18:0 | 0.17 | g | C-18:1 | 3.28 | g |
| C-18:2 | 3.11 | g | C-18:3 | 1.85 | g |
| C-20:0 | 0.04 | g | C-20:1 | 0.03 | g |
| Calories | 470 | | Calcium | 89 | mg |
| Carbohydrate | 55.67 | g | Calories from Fat | 175 | |
| Cholesterol | 0 | mg | Dietary Fiber | 26.5 | g |
| Iron | 88 | mg | Moisture | 3.54 | g |
| Insoluble Fiber | 25.2 | g | Monounsaturated Fat | 3.42 | g |
| Sodium | 540 | mg | Protein | 18.08 | g |
| Polyunsaturated Fat | 5.00 | g | Soluble Fiber | 1.3 | g |
| Sugars | 0.77 | g | Saturated Fat | 3.12 | g |
| Vitamin A | 22000 | IU | Vitamin C | 0.00 | mg |
| Calories from Saturated Fat | 28 | | | | |

All analytical results based on 100 grams with the exception of peroxide.

N.D. - not determined, color of sample interfered with analysis.

Ann Wallace
Chemistry Supervisor

Alex Krein
Instrumentation Supervisor

Warren Analytical Laboratory
P.O. Box G
650 "0" Street
Greeley, Colorado 80632-0350
(970) 351-6344/Fax: (970) 351-6648
1-800-945-6669

**LABORATORY ANALYSIS REPORT**
**TRADE SECRET**
**Confidential Commercial Report**

Attn: MIA UNSON
AQUASEARCH, INC.



Work Order No. 9914389 Flakes

Receive Date: 06/18/99 Reported: 06/18/99

| SMP. DATE | IDENTIFICATION | TYPE | I.D. |
|---|---|---|---|
| 06/16/99 | 990610 MIX H.PLUVIALIS FLAKES | Flakes | 99RB-02811 |

| | | | | | |
|---|---|---|---|---|---|
| Folic Acid | 0.39 | mg | Pantothenic Acid | 2.47 | mg |
| Vitamin B6 | 0.14 | mg | Vitamin B12 | 0.04 | mg |

NOTE: All analytical results based on 100 grams.

**Alex Krein**
**Instrumentation Supervisor**

**LABORATORY ANALYSIS REPORT**
**TRADE SECRET**
**Confidential Commercial Report**

Attn: MIA UNSON
AQUASEARCH, INC.

Page: 1

Work Order No. 9914353 Flakes

Receive Date: 06/18/99 Reported: 06/18/99

| SMP. DATE | IDENTIFICATION | TYPE | I.D. |
|---|---|---|---|
| 06/16/99 | 990610 MIX H PLUVIALIS FLAKES | Flakes | 99MB-42497 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Total Plate Count | <200 | CFU/g | Anaerobic Plate Count | 200 | CFU/g |
| Total Coliform Count | <10 | CFU/g | Escherichia coli Count | <10 | CFU/g |
| Yeast and Mold Count | 50 | CFU/g | Salmonella | Negative | /25g |

**Ed Grove**
**Microbiology Supervisor**

#2



**ASPLAND AND JAMES LIMITED**
**CONSULTANT ANALYSTS**
Medcalfe Way, Bridge Street, Chatteris,
Cambridgeshire, PE16 6QZ, UK.
Tel: +44 (0) 1354 695858
Fax: +44 (0) 1354 692215
E.mail: ajlabs@aol.com

# Certificate Of Analysis

**Mr M Guerin**
**Finnfeeds International Ltd**
**PO Box 777**
**Marlborough**
**Wiltshire**
**SN8 1XN**

**Certificate Dated** 30 October, 1998 **Date Received** 12/10/1998
**Certificate Number** 111943-1 Interim **Microbiology Started** 09/10/1998

| Our Ref | Sample Details | Test | Result | SOP No. |
|---|---|---|---|---|
| 221616 | Algae Meal<br>Lot No: HP980051 | Moisture % | 4.9 | AM/C/801/7 |
| | | Protein (Nx6.25) % | 21.1 | AM/C/224/6 |
| | | Oil (Acid Hydrolysis) % | 29.3 | AM/C/102/5 |
| | | Crude Fibre % | 2.5 | AM/C/301/6 |
| | | Ash % | 7.7 | AM/C/803/5 |
| | | Cadmium mg/kg | 0.05 | AM/C/604/8 |
| | | Lead mg/kg | 2.76 | AM/C/604/8 |
| | | Mercury# mg/kg | ND<0.01 | AM/C/606/2 |
| | | Arsenic mg/kg | 0.21 | AM/C/616/4 |
| | | Astaxanthin | TF | AM/C/943/2 |
| | | Mycotoxin screen (sub-contracted)# | ND | |
| | | TVC c.f.u. /g | $3.5x10^3$ | AM/M/312/4 |
| | | Coliforms c.f.u. /g | <10 | AM/M/311/4 |
| | | E.coli in 1g | Not Found | AM/M/304/5 |
| | | Salmonella in 2x25g | Not Found | AM/M/351/7 |

None of the mycotoxins covered by the screening procedure were detected. Those that would have been detected, if present are:-

| | **Limits of Detection** |
|---|---|
| Aflatoxin B1, B2, G1,G2 | 15 µg/kg |
| Ochratoxin | 20 µg/kg |
| Zearalenone | 500 µg/kg |
| Vomitoxin | 1000 µg/kg |
| T2 Toxin | 500 µg/kg |

00009-1

D Hall (Team Leader, Chemistry)

A Brack (Section Head, Microbiology)

GLP

Tests marked* in this certificate are not included in the UKAS Accreditation Schedule for our laboratory
Subjective comments are not covered by the scope of UKAS

# University of Hawai'i at Mānoa

College of Tropical Agriculture and Human Resources
Department of Animal Sciences
1800 East-West Road • Honolulu, Hawai'i 96822

August 1, 1995

MEMORANDUM

TO: Robert R. Bidigare
Department of Oceanography
University of Hawaii at Manoa

FROM: James R. Carpenter, Ruminant Nutritionist
Department of Animal Sciences

SUBJECT: Chemical Analysis of Freshwater Algal Material

The results of the chemical analysis of the freshwater algal material is shown below:

1. Dry matter and ash (AOAC procedure - 100°C for dry matter and 575°C for ash)

| crucible number | crucible weight | crucible & sample wt.(fresh) | crucible & sample wt.(dry) | crucible & sample wt. after ashing |
|---|---|---|---|---|
| 24 | 17.3408 g | 17.8390 g | 17.8175 g | 17.3860 g |

Weight of fresh sample: 17.8390 - 17.3408 = .4982 g
Weight of dry sample: 17.8175 - 17.3408 = .4767 g
% Dry matter: (.4767 / .4982) x 100 = 95.68%
Weight of ash: 17.3860 - 17.3408 = .0452 g
% Ash (dry matter basis): .0452 / .4767) x 100 = 9.48%
% Ash (fresh sample basis): .0452 / .4982) x 100 = 9.07%

2. Crude protein (AOAC Kjeldahl procedure, using % CP = % N x 6.25)

| paper weight | paper & fresh sample wt. | fresh sample weight | Normality of acid | ml of acid for titration | ml of acid reagent blank |
|---|---|---|---|---|---|
| .4171 g | 1.9174 g | 1.5003 g | .0956 | 40.35 ml | 0 ml |

Normality of acid x .014 = gms N equivalent to 1 ml of acid
Therefore: gms N equiv. = .0013384

% Nitrogen (DM basis): [((.0013384 x 40.35) / 1.5003) x 100] /.9568 = 3.762 % N
% Crude protein (dry matter basis): 3.762% N x 6.25 = 23.51 % CP
% Crude protein (fresh sample basis): 23.51% x .9568 = 22.49 % CP

000095

An Equal Opportunity/Affirmative Action Institution

3. Crude fat/lipid content (AOAC Goldfisch method using anhydrous ethyl ether)
Note: Ether extract also includes ether soluble pigments

| weight of thimble | weight of thimble & fresh sample | weight of fresh sample | weight of empty beaker | weight of beaker & ext. | wt. of extract |
|---|---|---|---|---|---|
| 12.3975 g | 13.3572 g | .9597 g | 67.9026 g | 68.1082 g | .2056 g |

% Crude fat/lipid (DM basis): .2056 / (.9597 x .9568) = 22.39%
% Crude ~~protein~~ fat/lipid (fresh sample basis): 22.39% x .9568 = 21.42%

The amount of sample used for each assay was based on your memorandum to me dated 17 July 1995. With the limited sample size only one determination was run for each nutrient class, but other samples (with known amounts of ash, crude protein and ether extract) were run simultaneously as controls.

3. Crude fat/lipid content (AOAC Goldfisch method using anhydrous ethyl ether)
   Note: Ether extract also includes ether soluble pigments

| weight of thimble | weight of thimble & fresh sample | weight of fresh sample | weight of empty beaker | weight of beaker & ext. | wt. of extract |
|---|---|---|---|---|---|
| 12.3975 g | 13.3572 g | .9597 g | 67.9026 g | 68.1082 g | .2056 g |

% Crude fat/lipid (DM basis): .2056 / (.9597 x .9568) = 22.39%
% Crude ~~protein~~ fat/lipid (fresh sample basis): 22.39% x .9568 = 21.42%

The amount of sample used for each assay was based on your memorandum to me dated 17 July 1995. With the limited sample size only one determination was run for each nutrient class, but other samples (with known amounts of ash, crude protein and ether extract) were run simultaneously as controls.

MARTIN-MIGUEL LATASA ARCALIS PhD
3029 Lowrey Avenue #2117
Honolulu, Hawaii 96822
(808) 988 4259

## REPORT

A sample with the description *Haematococcus* flakes was received for analysis of astaxanthin carotenoid content. The sample was a loose red powder/flakes.

*Sample preparation.*
Two subsamples were weighed with an error of less than 1%. Subsamples were ground in 100% acetone using a Potter-Elvehjem grinder. After grinding, the content was transferred to a centrifuge tube, spun for 5 minutes and the supernatant stored at 0°C in the dark. The pellet was then transferred to the grinder and re-extracted. This procedure was repeated 5-7 times to assure complete pigment extraction.

*Analysis*
200 µL of a mixture of 0.3 ml $H_2O$ plus 1.0 ml of the pooled extract were injected into a Varian 5000 HPLC system equipped with a Varian autosampler, a Timberline column heater set at 26°C, Radial-PAK $C_{18}$ column (100 x 8.0 mm, 5 µm particle size; Waters Chrom. Div.) ThermoSeparation UV2000 detector recording absorbance at 436 nm and 450 nm simultaneously (only chromatograms recorded at 450 nm were used for quantitative analysis), and ThermoSeparation FL2000 detector with excitation set at 410± 40 nm and emission collected between 600-800 nm (Omega Optical, custom filters). Separations were performed with a three solvent system: eluent A (methanol:0.5 M ammonium acetate 80:20), eluent B (methanol) and eluent C (ethyl acetate). The linear gradient used for pigment separation was a modified version of the Bidigare et al. (1993) method and it ran as follows: 0':100%A; 12':100%B; 24':100%B; 60':50%B/50%C. All solvents were HPLC grade (Fisher). The flow was held constant at 6 mL $min^{-1}$. Purified astaxanthin, chlorophyll *a*, chlorophyll *b* and β-carotene standards were used to quantify pigment concentrations. Subsamples were analyzed in duplicate.

## RESULTS

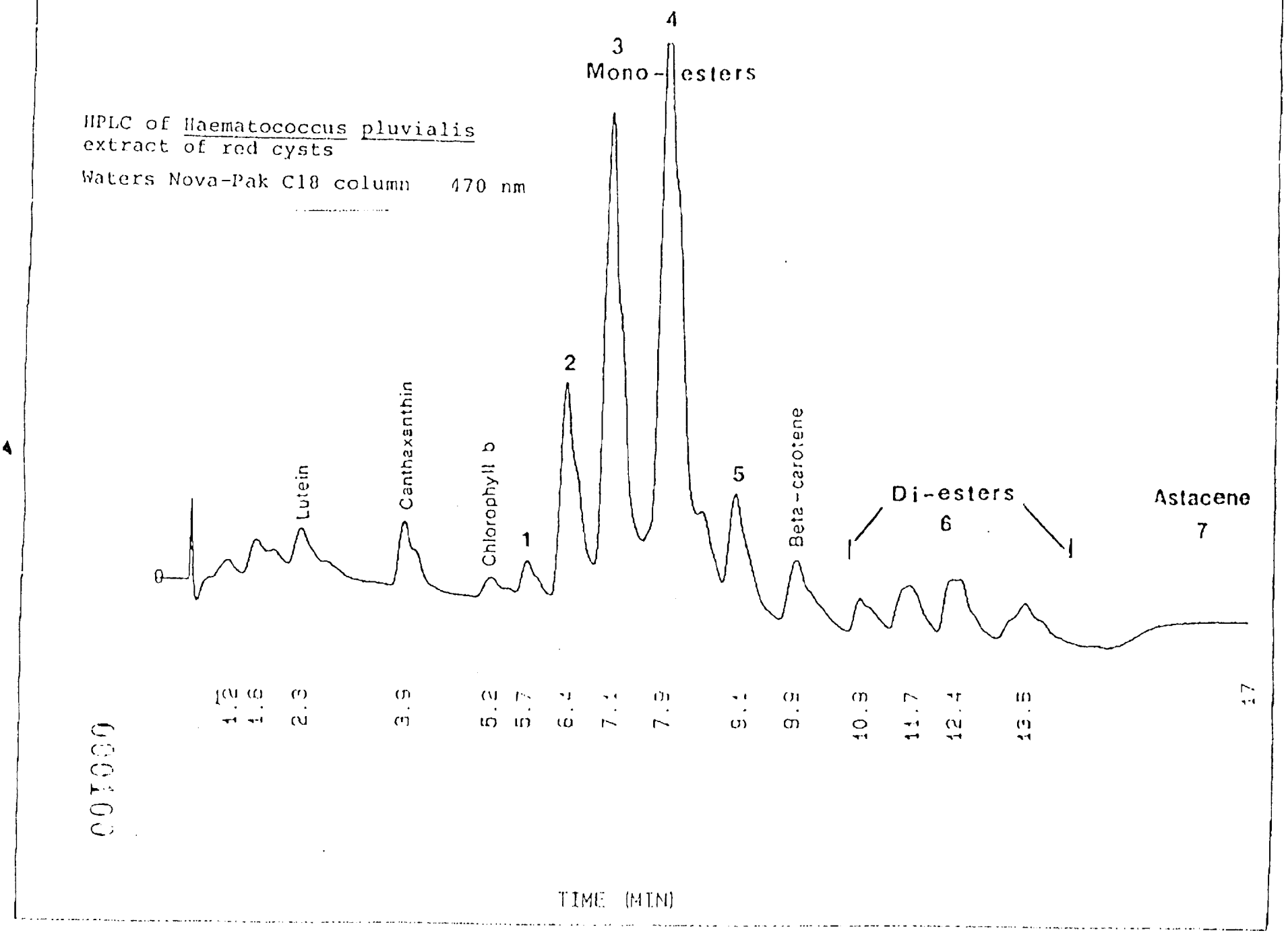
Pigment concentrations are summarized in the attached table. Astaxanthin composition was 10.3%:87.2%:2.5% diester:monoester:free astaxanthin.

## REFERENCES

Bidigare, R.R, M.E. Ondrusek, M.C. Kennicutt II, R. Iturriaga, H.R. Harvey, R.W. Hoham and S.A. Macko. 1993. Evidence for a photoprotecetive function for secondary carotenoids of snow algae. *Journal of Phycology*, 29, 427-434.

000093

| HPLC PIGMENT ANALYSIS (University of Hawaii): ng pigment/filter | | | | |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | % content |
| Dry weight (mg) | 2.5 | 2.5 | 2.5 | 100% |
| Astaxanthin | 94881 | 89425 | 92153 | 3.69% |
| Neoxanthin | 646 | 565 | 605.5 | 0.02% |
| Violaxanthin | 307 | 326 | 316.5 | 0.01% |
| Lutein | 3344 | 3661 | 3502.5 | 0.14% |
| alpha-Carotene | 0 | 0 | 0 | 0.00% |
| beta-Carotene | 0 | 0 | 0 | 0.00% |
| *Total carotenoids* | 99178 | 93977 | 96577.5 | 3.86% |
| | | | | |
| Chlorophyll a | 3990 | 5947 | 4968.5 | 0.20% |
| Chlorophyll b | 5452 | 6518 | 5985 | 0.24% |
| Chlorophyllide a | 548 | 431 | 489.5 | 0.02% |


HPLC of Haematococcus pluvialis
extract of red cysts
Waters Nova-Pak C18 column 470 nm
Lutein
Canthaxanthin
Chlorophyll b
1
2
3
Mono-esters
4
5
Beta-carotene
Di-esters
6
Astacene
7
4.6
4.8
2.3
3.9
5.2
6.2
9.9
10.3
11.7
12.4
13.5
17
TIME (MIN)

E. H. De CARLO Ph.D.
ANALYTICAL, ENVIRONMENTAL AND MARINE CONSULTANTS
2654 Lowrey Avenue
Honolulu, Hawaii 96822
(808)-988-5028

Narrative Report

Sample Description:

A sample of Haematococcus algae was received for analysis of a series of trace elements (See Table 1). Approximately 1.5 g of dried (ground up) algae was provided in a plastic bottle. The sample was dissolved with mineral acids prior to analysis without any further drying or other pre-treatment.

Sample Peparation:

Three splits of appproximately 0.5 g each were weighed exactly to the nearest 0.1 mg. Each of the three splits was transferred to a Teflon digestion vessel under a Class 1000 (clean air) laminar flow hood, 5 mL of Ultrex grade (ultra-high purity) $HNO_3$ was added to each vessel, and the reaction vessel sealed. The digestion vessels were palced in a microwave oven and heated to effectuate sample dissolution. A series of 10 minute heating cycles was employed with power increased gradually to ensure thorough and uniform heating of the samples. After digestion was completed the samples were transferred to (acid-washed/trace metal clean) 25-mL volumetric flasks nalgene bottles and the solutions made up to the mark with >17.5 Mohm-cm resistivity distilled deionized water. Samples were transferred to acid-cleaned Nalgene bottles for storage prior to analysis. The first sample split exhibited a substantial amount of fine grey particles after digestion that could not be brought into solution. This solution was subjected to analysis; results indicated that the sample was valid, but in light of the particulate residues, results from this split are not reported below.

Sample Analysis:

Digested sample solutions were initially analyzed by graphite furnace atomic absorption spectromety (GFAAS) and inductively coupled plasma-optical emission spectrometry, and the preliminary results subsequently verified by inductively coupled plasma-mass spectrometry (ICP/MS). The data in Table 1 represent results of the ICP-MS analysis. For ICP-MS analysis, 1 mL each of the digested solutions was diluted to 10 mL with distilled deionized water after addition of 10 µg/L indium as an internal standard. The solutions were aspirated into the instrument and the mass range of interest scanned after calibration of the instrument with multi-element aqueous standards ranging in concentration from 0-10 µg/L.



000101

Instrument signals were normalized to that of the internal standard to correct for instrument drift. Most elements were also analyzed at several isotopic masses to provide additional quality assurance and quality control. Several elements are subject to isobaric interferences from isotopes of other constituents; in such cases only the isobaric interference-free data are reported. Elemental concentrations are reported on an air-dried basis in the original sample material.

Special Notes:

Because the Haematococcus samples were collected under unknown conditions by persons unknown to us, no guarantee can be made that the concentrations measured in the samples provided to us represent levels of these elements actually present in Haematococcus algae. Contamination of samples containing low concentrations of heavy metals can occur readily and is likely when sample collection is performed by personnel not specifically trained in trace element clean techniques.



000102

Table 2

Carotenoid content and composition of three algal samples (Haematococcus) provided by Aquasearch

| Sample | HCS1 *) | HCS1 | HCS2 | HCS3 |
|---|---|---|---|---|
| Receipt | 4.9.91 | 4.9.91 | 11.9.91 | 25.9.91 |
| Lipids % of dry weight | 20 | | 5.4 | 26 |
| Chl a % " " " | 0.66 | | 0.22 | 0.58 |
| | 0.93 | → | 0.27 | 0.76 |
| Chl b % " " " | 0.27 | | 0.05 | 0.18 |
| Carotenoids | 1.64 (1.2%) | | 0.31 (0.2%) | 3.87 |
| Composition | % | % | % | % |
| Astaxanthin (D:M:F)*) | 70.0 (21:75:4) | 66.3 (21:76:3) | 51.7 (18:53:29) | 80.4 (30:69:1) |
| Adonirubin (E:F)**) | 5.4 (88:12) | 5.7 (88:12) | 4.4 (61:39) | 10.8 (94:6) |
| Echinenone | 2.5 | 2.1 | 2.2 | 0.9 |
| Canthaxanthin | 1.4 | 1.5 | 1.4 | 0.6 |
| β,β Carotene | 4.6 | 4.8 | 7.1 | 1.4 |
| Lutein | 10.0 | 11.7 | 22.9 | 4.1 |
| Zeaxanthin | 0.5 | 0.6 | 2.3 | 0.2 |
| α-Adonixanthin | 0.4 | 0.3 | 0 | trace |
| Xanthophyll epoxides | 1.4 | 1.4 | 0.8 | 0.3 |
| Polyhydroxy xanthoph. | 2.2 | 1.3 | 3.0 | 0.5 |
| n.i. | 1.6 | 4.3 | 4.5 | 0.5 |

*) analyzed with more detailed procedure (results presented in I.O.C. of Sept. 27, 1991)

**) D = diester, M = monoester F = free, E = ester

Sdt 207/16.10.91

E.H. De Carlo, Ph.D.
Analytical, Environmental & Marine Consultants
2654 Lowrey Avenue
Honolulu, Hawaii

*Haematococcus pluvialis* *
Elemental Analysis

| Element | Isotope Mass | Concentration (ug/g dry wt) Replicate #1 | Replicate #2 | Mean |
|---|---|---|---|---|
| Al | 27 | 387 | 388 | 387.5 |
| As | 75 | 0.548 | 0.662 | 0.605 |
| Bi | | 0.005 | 0.007 | 0.006 |
| Cd | 110 | 0.134 | 0.139 | 0.137 |
| Cd | 111 | 0.136 | 0.120 | 0.128 |
| Cd | 115 | 0.128 | 0.113 | 0.121 |
| Co | 59 | 2.89 | 2.92 | 2.91 |
| Cr | 52 | 9.53 | 9.46 | 9.50 |
| Fe | 57 | 1217 | 1252 | 1235 |
| Hg | | 0.00 | 0.00 | 0.00 |
| Mo | 95 | 2.08 | 2.11 | 2.10 |
| Ni | 60 | 6.52 | 6.90 | 6.71 |
| Pb | 206 | 4.81 | 4.96 | 4.89 |
| Pb | 207 | 3.83 | 4.03 | 3.93 |
| Pb | 208 | 4.23 | 4.44 | 4.34 |
| Se | 78 | 0.83 | 0.79 | 0.81 |
| Se | 82 | 0.64 | 0.84 | 0.74 |
| Sr | 86 | 29.0 | 28.7 | 28.9 |
| Sr | 88 | 29.2 | 28.8 | 29.0 |
| V | | 18.6 | 18.6 | 18.6 |
| W | 182 | 0.041 | 0.045 | 0.043 |
| W | 184 | 0.058 | 0.042 | 0.050 |
| Zn | 64 | 107 | 113 | 110 |
| Zn | 66 | 116 | 122 | 119 |

*Source: Aquasearch Inc.